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(54) Title: IL-17 LIKE MOLECULES AND USES THEREOF

(57) Abstract: Novel IL-17 like polypeptides and nucleic acid molecules encoding the same. The invention also provides vectors, host cells, selective binding agents, and methods for producing IL-17 like polypeptides. Also provided for are methods for the dignosis, treatment, or prevention of diseases with IL-17 like polypeptides or antagonists thereof.

IL-17 LIKE MOLECULES AND USES THEREOF

Related Application

This application claims priority from U.S. patent application serial no. 09/722,920 filed November 27, 2000 which claims priority from U.S. provisional patent application serial No. 60/180,864 filed February 8, 2000.

Field of the Invention

The present invention relates to novel IL-17 like polypeptides and nucleic acid molecules encoding the same. The invention also relates to vectors, host cells, pharmaceutical compositions, selective binding agents and methods for producing IL-17 like polypeptides. Also provided for are methods for the diagnosis and treatment of diseases associated with IL-17 like polypeptides.

Background of the Invention

Technical advances in the identification, cloning, expression and manipulation of nucleic acid have greatly accelerated the discovery of novel therapeutics based upon deciphering the human genome. Rapid nucleic acid sequencing techniques can now generate sequence information at unprecedented rates and, coupled with computational analyses, allow the assembly of overlapping sequences into partial and entire genomes and the identification of polypeptide-encoding regions. A comparison of a predicted amino acid sequence against a database compilation of known amino acid sequences can allow one to determine the extent of homology to previously identified sequences and/or structural landmarks. The cloning and expression of a polypeptide-encoding region of a nucleic acid molecule provides a polypeptide product for structural and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides to create variants and derivatives thereof may confer advantageous properties on a product for use as a therapeutic.

In spite of the significant technical advances in genome research over the past decade, the potential for the development of novel therapeutics based on the human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics, or those encoding polypeptides, which may act as "targets" for therapeutic molecules, have still

not been identified. In addition, structural and functional analyses of polypeptide products from many human genes have not been undertaken.

IL-17 is an activated T cell derived cytokine. IL-17 has been found to play a regulatory role in inflammation by inducing expression of pro-inflammatory cytokines. Recent studies reveal that it may also be involved in bone destruction by affecting osteoclasic resorption.

Summary of the Invention

The present invention relates to novel IL-17 like nucleic acid molecules and encoded polypeptides. .

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2;

(c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b) wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2; and

(d) a nucleotide sequence complementary to any of (a)-(c).

The invention also provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NO: 2, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 as determined using a computer program such as GAP, BLASTP, BLASTN, BLASTA, BLASTX, BestFit, or the Smith-Waterman algorithm;

(b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NO: 1, wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(c) a nucleotide sequence of SEQ ID NO: 1, (a), or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(d) a nucleotide sequence of SEQ ID NO: 1, or (a)-(c) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d), wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2; and

(f) a nucleotide sequence complementary to any of (a)-(c).

The invention further provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(f) a nucleotide sequence of (a)-(e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2; and

(h) a nucleotide sequence complementary to any of (a)-(e).

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) an amino acid sequence comprising the mature IL-17 like polypeptide as depicted by amino acid residue 5 through amino acid residue 227 of SEQ ID NO: 2 and optionally further comprising an amino-terminal methionine;

(b) an amino acid sequence for an ortholog of SEQ ID NO: 2;

(c) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 2, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 as determiuned using a computer program such as GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algoarithm;

(d) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(e) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NO: 2, or at least one of (a)-(c), wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2.

Additionally, the invention provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(b) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(c) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(d) the amino acid sequence as set forth in SEQ ID NO: 2 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2; and

(e) the amino acid sequence as set forth in SEQ ID NO: 2, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2.

Also provided are fusion polypeptides comprising the amino acid sequences of (a)-(g) above.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein, recombinant host cells comprising recombinant nucleic acid molecules as set forth herein, and a method of producing an IL-17 like polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced. These expression vectors include baculovirus expression vectors which utilize insect cells for expression.

A transgenic non-human animal comprising a nucleic acid molecule encoding an IL-17 like polypeptide is also encompassed by the invention. The IL-17 like nucleic acid molecules are introduced into the animal in a manner that allows expression and increased levels of an IL-17 like polypeptide, which may include increased circulating levels. The transgenic non-human animal is preferably a mammal. Also provided is a transgenic non-human animal comprising a disruption in the nucleic acid molecule encoding a IL-17 like polypeptide, which will knock-out or significantly decrease expression of the IL-17 like polypeptide.

Also provided are derivatives of the IL-17 like polypeptides of the present invention.

Analogs of the IL-17 like polypeptides are provided for in the present invention which result from conservative and/or non-conservative amino acids substitutions of the IL-17 like polypeptide of SEQ ID NO: 2. Such analogs include an IL-17 like polypeptide wherein, for

example the amino acid at position 47 of SEQ ID NO: 2 is leucine, norleucine, isoleucine, valine, methionine, alanine or phenylalanine, the amino acid at position 110 of SEQ ID NO: 2 is gluatamic acid or aspartic acid, the amino acid at position 141 of SEQ ID NO: 2 is tyrosine, tryptophan, phenylalanine, threonine or serine, the amino acid at position 151 of SEQ ID NO: 2 is proline, alanine or glycine, the amino acid at position 159 of SEQ ID NO: 2 is cysteine, alanine or serine, the amino acid at position 161 of SEQ ID NO: 2 is cysteine, alanine or serine, the amino acid at position 164 of SEQ ID NO: 2 is cysteine, alanine or serine, the amino acid at position 193 of SEQ ID NO: 2 is cysteine, alanine or serine, the amino acid at position 219 of SEQ ID NO: 2 is cysteine, alanine or serine, or the amino acid at position 221 of SEQ ID NO: 2 is cysteine, alanine or serine.

Additionally provided are selective binding agents such as antibodies and peptides capable of specifically binding the IL-17 like polypeptides of the invention. Such antibodies, polypeptides, peptides and small molecules may be agonistic or antagonistic.

Pharmaceutical compositions comprising the nucleotides, polypeptides, or selective binding agents of the present invention and one or more pharmaceutically acceptable formulation agents are also encompassed by the invention. The pharmaceutical compositions are used to provide therapeutically effective amounts of the nucleotides or polypeptides of the present invention. The invention is also directed to methods of using the polypeptides, nucleic acid molecules and selective binding agents.

The IL-17 like polypeptides and nucleic acid molecules of the present invention may be used to treat, prevent, ameliorate, diagnose and/or detect diseases and disorders, including those recited herein. Expression analysis in biological, cellular or tissue samples suggests that IL-17 like polypeptide may play a role in the diagnosis and/or treatment of the pathological conditions described herein. This expression can be detected with a diagnostic agent such as a IL-17 like polynucleotide.

The invention encompasses diagnosing a pathological condition or the susceptibility to a pathological condition in a subject caused by or resulting from abnormal (i.e. increaded or decreased) levels of IL-17 like polypeptide comprising determining the presence or amount of expression of the IL-17 like polypeptide in a sample and comprising the level of said polypeptide in a biological, tissue or cellular sample from either normal subjects or the

subject at an earlier time, wherein susceptibility to a pathological condition is based on the presence or amount of expression of the polypeptide.

The present invention also provides a method of assaying test molecules to identify a test molecule which binds to an IL-17 like polypeptide. The method comprises contacting an IL-17 like polypeptide with a test molecule and determining the extent of binding of the test molecule to the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists of an IL-17 like polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of IL-17 like polypeptide or on the activity of IL-17 like polypeptide.

The present invention provides for methods of identifying antagonists or agonists of IL-17 like biological activity comprising contacting a small molecule compound with IL-17 like polypeptides and measuring IL-17 like biological activity in the presence and absence of these small molecules. These small molecules can be a naturally occurring medicinal compound or derived from combinational chemical libraries. In certain embodiments, an IL-17 like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecule which interacts with a IL-17 like polypeptide to regulate its activity.

Methods of regulating expression and modulating (i.e., increasing or decreasing) levels of an IL-17 like polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule encoding an IL-17 like polypeptide. In another method, a nucleic acid molecule comprising elements that regulate or modulate the expression of an IL-17 like polypeptide may be administered. Examples of these methods include gene therapy, cell therapy and anti-sense therapy as further described herein.

In another aspect of the present invention, the IL-17 like polypeptides may be used for identifying binding partners thereof ("IL-17 like polypeptide receptors"). Yeast two-hybrid screens have been extensively used to identify and clone receptors for protein ligands. (Chien et al., Proc. Natl. Acad. Sci. U.S.A., 88: 9578-9583, 1991). The isolation of a IL-17 like polypeptide binding partner is useful for identifying or developing novel agonists and antagonists of the IL-17 like polypeptide activity. Such agonists and antagonists include soluble anti-IL-17 like receptor(s), anti-IL-17 like selective binding agents and/or anti-IL-17 like receptor selective binding agents (such as antibodies and derivatives thereof), small

molecules, peptides or derivatives thereof capable of binding IL-17 like polypeptide or antisense oligonucleotides, any of which can be used for potentially treating one or more diseases or disorders disclosed, including those recited herein.

The invention further encompasses methods for determine the presence of IL-17 like nucleic acids in a biological, tissue or cellular sample . These methods comprise the steps of providing a biological sample suspected of containing IL-17 like nucleic acids; contacting the biological sample with a diagnostic reagent of the present invention under conditions wherein the diagnostic reagent will hybridize with IL-17 like nucleic acids contained in said biological sample; detecting hybridization between nucleic acid in the biological sample and the diagnostice reagent; and comparing the level of hybridization between the biological sample and diagnostic reagent with the level of hybridization between a known concentration of IL-17 like nucleic acid and the diagnostic reagent. The polynucleotide detected in these methods may be an IL-17 like DNA or and IL-17 like RNA.

The invention also provides for a device which comprises a membrane suitable for implantation in a pateint; and cells encapsulated within said membrane, wherein said cells secrete an IL-17 like polypeptide of the invnetion wherein the membrane is permeable to the protein product and impermeable to materials detrimental to said cells. The invention further provides for a device which comprises a membrane suitable for implantation and the IL-17 like polypeptide encapsulated in a membrane that is permeable to the polypeptide

Brief Description of the Figures

Figure 1 depicts a nucleic acid sequence (SEQ ID NO:1) encoding the human IL-17 like molecule. Also depicted is the amino acid sequence, (SEQ ID NO: 2) of the human IL-17 like polypeptide.

Figure 2 depicts an amino acid sequence (SEQ ID NO: 3) for the human IL-17 like molecule, wherein the predicted amino terminal signal peptide sequence is underlined.

Figure 3 (SEQ ID NO: 4) depicts an overlap of the human IL-17 like amino acid sequence with the known amino acid sequence of human IL-17.

Detailed Description of the Invention

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described therein. All references cited in this application are expressly incorporated by reference herein.

Definitions

The terms "IL-17 like gene" or "IL-17 like nucleic acid molecule" or "IL-17 like polynucleotide" refers to a nucleic acid molecule comprising or consisting of a nucleotide sequence as set forth in SEQ ID NO: 1, a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2, a nucleotide sequence of the DNA insert in ATCC deposit no. PTA-1451 (deposited with the American Type Culture Collection 10801 University Blvd. Manassas, VA on March 7, 2000) or related nucleic acid molecules as defined herein.

The term "IL-17 like polypeptide" refers to a polypeptide comprising the amino acid sequence of at least one of SEQ ID NO: 2 or SEQ ID NO: 3, and related polypeptides. Related polypeptides include: IL-17 like polypeptide allelic variants, IL-17 like polypeptide orthologs, IL-17 like polypeptide splice variants, IL-17 like polypeptide variants and IL-17 like polypeptide derivatives. IL-17 like polypeptides may be mature polypeptides, as defined herein, and may or may not have an amino terminal methionine residue, depending on the method by which they are prepared.

The term "IL-17 like polypeptide allelic variant" refers to one of several possible naturally occurring alternate forms of a gene occupying a given locus on a chromosome of an organism or a population of organisms.

The term "IL-17 like polypeptide derivatives" refers to the polypeptide as set forth in SEQ ID NO: 2, IL-17 like polypeptide allelic variants, IL-17 like polypeptide fragments, IL-17 like polypeptide orthologs, IL-17 like polypeptide splice variants, or IL-17 like polypeptide variants, as defined herein, that have been chemically modified. The derivatives are modified in a manner that is different from naturally occurring IL-17 like polypeptides, either in the type or location of the molecules attached to the polypeptide. Derivatives may

further include molecules formed by the deletion of one or more chemical groups which are naturally attached to the IL-17 like polypeptide.

The term "IL-17 like polypeptide fragment" refers to a polypeptide that comprises a truncation at the amino terminus (with or without a leader sequence) and/or a truncation at the carboxy terminus of the polypeptide as set forth in SEQ ID NO: 2, IL-17 like polypeptide allelic variants, IL-17 like polypeptide orthologs, IL-17 like polypeptide splice variants and/or an IL-17 like polypeptide variant having one or more amino acid additions or substitutions or internal deletions (wherein the resulting polypeptide is at least 6 amino acids or more in length) as compared to the IL-17 like polypeptide amino acid sequence set forth in SEQ ID NO: 2. IL-17 like polypeptide fragments may result from alternative RNA splicing or from *in vivo* protease activity. In preferred embodiments, truncations comprise about 10 amino acids, or about 20 amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or more than about 100 amino acids. The polypeptide fragments so produced will comprise about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 150 amino acids, or about 200 amino acids. Such IL-17 like polypeptide fragments may optionally comprise an amino terminal methionine residue. It will be appreciated that such fragments can be used, for example, to generate antibodies to IL-17 like polypeptides.

The term "IL-17 like fusion polypeptide" refers to a fusion of one or more amino acids (such as a heterologous peptide or polypeptide) at the amino or carboxy terminus of the polypeptide as set forth in SEQ ID NO: 2, IL-17 like polypeptide allelic variants, IL-17 like polypeptide orthologs, IL-17 like polypeptide splice variants, or IL-17 like polypeptide variants having one or more amino acid deletions, substitutions or internal additions as compared to the IL-17 like polypeptide amino acid sequence set forth in SEQ ID NO: 2.

The term "IL-17 like polypeptide ortholog" refers to a polypeptide from another species that corresponds to IL-17 like polypeptide amino acid sequence as set forth in SEQ ID NO: 2. For example, mouse and human IL-17 like polypeptides are considered orthologs of each other.

The term "IL-17 like polypeptide splice variant" refers to a nucleic acid molecule, usually RNA, which is generated by alternative processing of intron sequences in an RNA transcript of IL-17 like polypeptide amino acid sequence as set forth in SEQ ID NO: 2.

The term "IL-17 like polypeptide variants" refers to IL-17 like polypeptides comprising amino acid sequences having one or more amino acid sequence substitutions, deletions (such as internal deletions and/or IL-17 like polypeptide fragments), and/or additions (such as internal additions and/or IL-17 like fusion polypeptides) as compared to the IL-17 like polypeptide amino acid sequence set forth in SEQ ID NO: 2 (with or without a leader sequence). Variants may be naturally occurring (*e.g.*, IL-17 like polypeptide allelic variants, IL-17 like polypeptide orthologs and IL-17 like polypeptide splice variants) or artificially constructed. Such IL-17 like polypeptide variants may be prepared from the corresponding nucleic acid molecules having a DNA sequence that varies accordingly from the DNA sequence as set forth in SEQ ID NO: 2. In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, wherein the substitutions may be conservative, or non-conservative, or any combination thereof.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes. The specific binding reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens.

The terms "biologically active IL-17 like polypeptides", "biologically active IL-17 like polypeptide fragments", "biologically active IL-17 like polypeptide variants", and "biologically active IL-17 like polypeptide derivatives" refer to IL-17 like polypeptides having at least one activity characteristic of a IL-17 like polypeptide, such as the activity of the polypeptide set forth in either SEQ ID NO: 2 or SEQ ID NO: 4. In general, IL-17 like

polypeptides, fragments, variants, and derivatives thereof, will have at least one activity characteristic of a IL-17 like polypeptide such as depicted in either SEQ ID NO: 2 or SEQ ID NO: 4. In addition, a IL-17 like polypeptide may be active as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised.

The terms "effective amount" and "therapeutically effective amount" refer to the amount of a IL-17 like polypeptide or IL-17 like nucleic acid molecule used to support an observable level of one or more biological activities of the IL-17 like polypeptides as set forth herein.

The term "expression vector" refers to a vector which is suitable for use in a host cell and contains nucleic acid sequences which direct and/or control the expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

The term "identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms").

The term "similarity" is a related concept, but in contrast to "identity", refers to a measure of similarity which includes both identical matches and conservative substitution matches. If two polypeptides have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity

would both be 50%. If in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the per cent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the invention that (1) has been separated from at a least about 50 percent of proteins, lipids , carbohydrates or other materials with which it is naturally found when total DNA is isolated for the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from at least one contaminating nucleic acid molecule with which it is naturally associated. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment which would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been sperated from at least about 50 percent of polynucleotides, lipids, carbohydrates or other materials with which it is naturally found when isolated from the cell source, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked to in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably is free from at least one contaminating polypeptide or other contaminants that are found in its natural environment. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment which would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "mature IL-17 like polypeptide" refers to an IL-17 like polypeptide lacking a leader sequence. A mature IL-17 like polypeptide may also include other modifications such

as protcolytic processing of the amino terminus (with or without a leader sequence) and/or the carboxy terminus, cleavage of a smaller polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and the like. An exemplary mature IL-17 like polypeptide is depicted by amino acid residue 45 through amino acid residue 223 of SEQ ID NO: 3.

The term "nucleic acid sequence" or "nucleic acid molecule" refers to a DNA or RNA sequence. The term encompasses molecules formed from any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-cytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxy-methylaminomethyluracil, dihydrouracil, inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5' -methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for

example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of the IL-17 like polypeptide, IL-17 like nucleic acid molecule or IL-17 like selective binding agent as a pharmaceutical composition.

The term "selective binding agent" refers to a molecule or molecules having specificity for an IL-17 like polypeptide. Selective binding agents include antibodies, such as polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, CDR-grafted antibodies, anit-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound forms, as well as fragments , regions, or derivatives thereof which are provided by known techniques , including, but not limited to enzymatic cleavage, peptide synthesis, or recombinant techniques. The anti-IL-17 like selective binding agents of the present invention are capable, for example, of binding portions of IL-17 like molecules to IL-17 like receptors.

As used herein, the terms, "specific" and "specificity" refer to the ability of the selective binding agents to bind to human IL-17 like polypeptides and not to bind to human non-IL-17 like polypeptides. It will be appreciated, however, that the selective binding agents may also bind orthologs of the polypeptide as set forth in SEQ ID NO: 2, that is, interspecies versions thereof, such as mouse and rat polypeptides.

IL-17 like polypeptides, fragments, variants, and derivatives may be used to prepare IL-17 like selective binding agents using methods known in the art. Thus, antibodies and antibody fragments that bind IL-17 like polypeptides are within the scope of the present invention. Antibody fragments include those portions of the antibody which bind to an epitope on the IL-17 like polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding

fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions. These antibodies may be, for example, polyclonal monospecific polyclonal, monoclonal, recombinant, chimeric, humanized, human, single chain, and/or bispecific.

The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, for example, Graham et al., Virology, 52:456 (1973); Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratories (New York, 1989); Davis et al., Basic Methods in Molecular Biology, Elsevier, 1986; and Chu et al., Gene, 13:197 (1981). Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, for example, Graham et al., Virology, 52:456 (1973); Sambrook et al., Molecular Cloning, a laboratory Manual, Cold Spring Harbor Laboratories (New York, 1989); Davis et al., Basic Methods in Molecular Biology, Elsevier, 1986; and Chu et al., Gene, 13:197 (1981). Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

Relatedness of Nucleic Acid Molecules and/or Polypeptides

It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecule of SEQ ID NO:1, and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/or a deletion of one or more amino acid residues compared to the polypeptide in SEQ ID NO. 2.

Fragments include molecules which encode a polypeptide of at least about 25 amino acid residues, or about 50, or about 75, or about 100, or greater than about 100 amino acid residues of the polypeptide of SEQ ID NO: 2.

In addition, related IL-17 like nucleic acid molecules include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully complementary sequence of the nucleic acid molecule of SEQ ID NO: 1, or of a molecule encoding a polypeptide, which polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 2, or of a nucleic acid fragment as defined herein, or of a nucleic acid fragment encoding a polypeptide as defined herein. Hybridization probes may be prepared using the IL-17 like sequences provided herein to screen cDNA, genomic or synthetic DNA libraries for related sequences. Regions of the DNA and/or amino acid sequence of IL-17 like polypeptide that exhibit significant identity to known sequences are readily determined using sequence alignment ' as described herein and those regions may be used to design probes for screening.

The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to

exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.015M sodium chloride, 0.0015M sodium citrate at 65-68oC or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42oC. See Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989); Anderson et al., Nucleic Acid Hybridisation: a practical approach, Ch. 4, IRL Press Limited (Oxford, England).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate (NaDodSO4 or SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson et al., Nucleic Acid

Hybridisation: a Practical Approach, Ch. 4, IRL Press Limited (Oxford, England).

Factors affecting the stability of a DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

$$Tm(oC) = 81.5 + 16.6(\log[Na+]) + 0.41(\%G+C) - 600/N - 0.72(\%formamide)$$

where N is the length of the duplex formed, [Na+] is the molar concentration of the sodium ion in the hybridization or washing solution, %G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1oC for each 1% mismatch.

The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015M sodium chloride, 0.0015M sodium citrate at 50-65oC or 0.015M sodium chloride, 0.0015M sodium citrate, and 20% formamide at 37-50oC. By way of example, a "moderately stringent" condition of 50oC in 0.015 M sodium ion will allow about a 21% mismatch.

It will be appreciated by those skilled in the art that there is no absolute distinction between "highly" and "moderately" stringent conditions. For example, at 0.015M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71oC. With a wash at 65oC (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences. one skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1M NaCl* for oligonucleotide probes up to about 20nt is given by:

Tm = 2oC per A-T base pair + 4oC per G-C base pair

*The sodium ion concentration in 6X salt sodium citrate (SSC) is 1M. See Suggs et al., Developmental Biology Using Purified Genes, p. 683, Brown and Fox (eds.) (1981).

High stringency washing conditions for oligonucleotides are usually at a temperature of 0-5oC below the Tm of the oligonucleotide in 6X SSC, 0.1% SDS.

In another embodiment, related nucleic acid molecules comprise or consist of a nucleotide sequence that is about 70 percent identical to the nucleotide sequence as shown in SEQ ID NO: 1, or comprise or consist essentially of a nucleotide sequence encoding a polypeptide that is about 70 percent identical to the polypeptide as set forth in SEQ ID NO: 2. In preferred embodiments, the nucleotide sequences are about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequence as shown in SEQ ID NO: 1, or the nucleotide sequences encode a polypeptide that is about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the polypeptide sequence as set forth in SEQ ID NO: 2.

Differences in the nucleic acid sequence may result in conservative and/or non-conservative modifications of the amino acid sequence relative to the amino acid sequence of SEQ ID NO: 2.

Conservative modifications to the amino acid sequence of SEQ ID NO: 2 (and the corresponding modifications to the encoding nucleotides) will produce IL-17 like polypeptides having functional and chemical characteristics similar to those of naturally occurring IL-17 like polypeptide. In contrast, substantial modifications in the functional and/or chemical characteristics of IL-17 like polypeptides may be accomplished by selecting substitutions in the amino acid sequence of SEQ ID NO: 2 that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the IL-17 like polypeptide, or to increase or decrease the affinity of the IL-17 like polypeptides described herein.

Exemplary amino acid substitutions are set forth in Table I.

Table I

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties. It will be appreciated by those of skill in the art that nucleic acid and polypeptide molecule described herein may be chemically synthesized as well as produced by recombinant means.

Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human IL-17 like polypeptide that are homologous with non-human IL-17 like polypeptide orthologs, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., J. Mol. Biol., 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose

hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in SEQ ID NO. 2 using well known techniques. For example, one may predict suitable areas of the molecule that may be changed without destroying biological activity. Also, one skilled in the art will realize that even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of an IL-17 like polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of an IL-17 like polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of the IL-17 like polypeptide. One skilled in the art

would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

For predicting suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of IL-17 like polypeptide to such similar polypeptides. After making such a comparison, one skilled in the art can determine residues and portions of the molecules that are conserved among similar polypeptides. One skilled in the art would know that changes in areas of the IL-17 like molecule that are not conserved would be less likely to adversely affect the biological activity and/or structure of a IL-17 like polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions).

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in an IL-17 like polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of IL-17 like polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of an IL-17 like polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using

acti ity assays know to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

Numerous scientific publications have been devoted to the prediction of secondary structure, and to the identification of epitopes, from analyses of amino acid sequences. See Chou et al., Biochemistry, 13(2):222-245 (1974); Chou et al., Biochemistry, 113(2):211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148 (1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting antigenic portions and epitopic core regions of proteins. Examples include those programs based upon the . Examples include those programs based upon the Jameson-Wolf analysis (Jameson et al., Comput. Appl. Biosci., 4(1):181-186 (1998) and Wolf et al., Comput. Appl. Biosci., 4(1):187-191 (1988), the program PepPlot® (Brutlag et al., CABS, 6:237-245 (1990), and Weinberger et al., Science, 228:740-742 (1985), and other new programs for protein tertiary structure prediction (Fetrow et al., Biotechnology, 11:479-483 (1993).

Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gainbecome dramatically in accuracy.more accurate.

Additional methods of predicting secondary structure include "threading""threading" (Jones. D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-9 (1996)), "profile analysis"4(1):15-19 (1996)). "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Home, supra,"evolutionary linkage" (See Holm, supra (1999), and Brenner, supra).

IL-17 like polypeptide analogs of the invention can be determined by comparing the amino acid sequence of IL-17 like polypeptide with related family members. An exemplary IL-17 like polypeptide related family member is human IL-17 polypeptide. This comparison can be accomplished by using a Pileup alignment (Wisconsin GCG Program Package) or an equivalent (overlapping) comparison with multiple family members within conserved and non-conserved regions.

As shown in Figure 3, the predicted amino acid sequence of human IL-17 like polypeptide (which represent amino acid 5 to 227 of SEQ ID NO: 2) is aligned with a known human IL-17 family member (SEQ ID NO: 4). Other IL-17 like polypeptide analogs can be determined using these or other methods known to those of skill in the art. These overlapping sequences provide guidance for conservative and non-conservative amino acids substitutions resulting in additional IL-17 like analogs. It will be appreciated that these amino acid substitutions can consist of naturally occurring or non-naturally occurring amino acids. For example, as depicted in Figure 3, alignment of the of related family members indicates potential IL-17 like analogs may have the Leu residue at position 47 of SEQ ID NO: 2 (position 42 on Fig. 3) substituted with a norleucine, Ile, Val, Met, Ala, or Phe residue, the Glu residue at position 110 of SEQ ID NO: 2 (position 106 on Fig. 3) substituted with a Asp residue, and/or the Tyr residue at position 141 of SEQ ID NO: 2 (position 137 on Fig. 3) substituted with a Trp, Phe, Thr, or Ser residue. In addition, potential IL-17 like analogs may have the Pro residue at position 151 of SEQ ID NO: 2 (position 147 on Fig. 3) substituted with a Ala or Gly residue, the Cys residue at position 159 of SEQ ID NO: 2 (position 155 on Fig. 3) substituted with a Ser or Ala residue, the Cys residue at position 161 of SEQ ID NO: 2 (position 157 on Fig. 3) substituted with a Ser or Ala residue, the Cys residue at position 164 of SEQ ID NO: 2 (position 160 on Fig. 3) substituted with a Ser or Ala residue, the Cys residue at position 193 of SEQ ID NO: 2 (position 189 on Fig. 3)

substituted with a Ser or Ala residue, the Cys residue at position 219 of SEQ ID NO: 2 (position 216 on Fig. 3) substituted with a Ser or Ala residue, and/or the Cys residue at position 221 of SEQ ID NO: 2 (position 218 on Fig. 3) substituted with a Ser or Ala residue.

In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, wherein the substitutions may be conservative, as described herein, or non-conservative, or any combination thereof. In addition, the variants can have additions of amino acid residues either at the carboxy terminus or at the amino terminus (with or without a leader sequence).

Preferred IL-17 like polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites has been altered compared to the native IL-17 like polypeptide. In one embodiment, IL-17 like polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution(s) of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred IL-17 like variants include cysteine variants, wherein one or more cysteine residues are deleted from or substituted with another amino acid (e.g., serine) Cysteine variants are useful when IL-17 like polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In addition, the polypeptide comprising the amino acid sequence of SEQ ID NO. 2 or an IL-17 like polypeptide variant may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of an IL-17 like fusion polypeptide; a transmembrane receptor protein or a portion

thereof, such as an extracellular domain, or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the IL-17 like polypeptide. In addition, a IL-17 like polypeptide may be fused to itself or to a fragment, variant, or derivative thereof.

Fusions can be made either at the amino terminus or at the carboxy terminus of an IL-17 like polypeptide. Fusions may be direct with no linker or adapter molecule or may be through using a linker or adapter molecule such as one or more amino acid residues up to about 20 amino acid residues , or up to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

In a further embodiment of the invention, an IL-17 like polypeptide variant, including a fragment, variant, and/or derivative, is fused to an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain known as "Fc", which links to such effector functions as complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas an Fab is short-lived. Capon et al., Nature, 337:525-31 (1989). When constructed together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein A binding, complement fixation and perhaps even placental transfer. Id. Table II summarizes the use of certain Fc fusions known in the art, including materails and methods applicable to the production of fused IL-17 like polypeptide.

Fc Fusion with Therapeutic Proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fcγ2a	IL-10	anti-inflammatory; transplant rejection	Zheng *et al.* (1995), *J. Immunol.*, 154: 5590-5600
IgG1	TNF receptor	septic shock	Fisher *et al.* (1996), *N. Engl. J. Med.*, 334: 1697-1702; Van Zee *et al.*, (1996), *J. Immunol.*, 156: 2221-2230
IgG, IgA, IgM, or IgE (excluding the first domain)	TNF receptor	inflammation, autoimmune disorders	U.S. Pat. No. 5,808,029, issued September 15, 1998
IgG1	CD4 receptor	AIDS	Capon *et al.* (1989), *Nature* 337: 525-531
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill *et al.* (1995), *Immunotech.*, 1: 95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614, published July 3, 1997
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig Cγ1	CTLA-4	autoimmune disorders	Linsley (1991), *J. Exp. Med.*, 174:561-569

In one example, all or a portion of the human IgG hinge, CH2 and CH3 regions may be fused at either the N-terminus or C-terminus of the IL-17 like polypeptides using methods known to the skilled artisan. In another example, a portion of hinge regions and CH2 and CH3 regions may be fused. The resulting IL-17 like polypeptide Fc-fusion polypeptide may be purified by use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life in vivo than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered

to improve certain qualities, such as therapeutic qualities, circulation time, reduce aggregation, etc.

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., SIAM J. Applied Math., 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res., 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol., 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, MD 20894; Altschul et al., supra). The well known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids

(the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff et al., Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff et al., Proc. Natl. Acad. Sci USA, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following:

Algorithm: Needleman et al., J. Mol. Biol., 48:443-453 (1970);

Comparison matrix: BLOSUM 62 from Henikoff et al., Proc. Natl. Acad. Sci. USA, 89:10915-10919 (1992);

Gap Penalty: 12

Gap Length Penalty: 4

Threshold of Similarity: 0

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparisons include the following:

Algorithm: Needleman et al., J. Mol Biol., 48:443-453 (1970);

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used, by those of skill in the art, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

Synthesis

It will be appreciated by those skilled in the art the nucleic acid and polypeptide molecules described herein may be produced by recombinant and other means.

Nucleic Acid Molecules

The nucleic acid molecules encode a polypeptide comparing the amino acid sequence of an IL-17 like polypeptide can readily be obtained in a variety of ways including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening and/or PCR amplification of cDNA.

Recombinant DNA methods used herein are generally those set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and/or Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, NY (1994). The present invention provides for nucleic acid molecules as described herein and methods for obtaining the molecules.

A gene or cDNA encoding a IL-17 like polypeptide or fragment thereof may be obtained by hybridization screening of a genomic or cDNA library, or by PCR amplification. Where a gene encoding the amino acid sequence of an IL-17 like polypeptide has been identified from one species, all or a portion of that gene may be used as a probe to identify corresponding genes from other species (orthologs) or related genes from the same species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the IL-17 like polypeptide. In addition, part or all of a nucleic acid

molecule having the sequence as set forth in SEQ ID NO: 1 may be used to screen a genomic library to identify and isolate a gene encoding the amino acid sequence of an IL-17 like polypeptide. Typically, conditions of moderate or high stringency will be employed for screening to minimize the number of false positives obtained from the screen.

Nucleic acid molecules encoding the amino acid sequence of IL-17 like polypeptides may also be identified by expression cloning which employs the detection of positive clones based upon a property of the expressed protein. Typically, nucleic acid libraries are screened by the binding of an antibody or other binding partner (*e.g.*, receptor or ligand) to cloned proteins which are expressed and displayed on a host cell surface. The antibody or binding partner is modified with a detectable label to identify those cells expressing the desired clone.

Recombinant expression techniques conducted in accordance with the descriptions set forth below may be followed to produce these polynucleotides and to express the encoded polypeptides. For example, by inserting a nucleic acid sequence which encodes the amino acid sequence of an IL-17 like polypeptide into an appropriate vector, one skilled in the art can readily produce large quantities of the desired nucleotide sequence. The sequences can then be used to generate detection probes or amplification primers. Alternatively, a polynucleotide encoding the amino acid sequence of an IL-17 like polypeptide can be inserted into an expression vector. By introducing the expression vector into an appropriate host, the encoded IL-17 like polypeptide may be produced in large amounts.

Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically complementary to two separate regions of cDNA (oligonucleotides) encoding the amino acid sequence of an IL-17 like polypeptide, are then added to the cDNA along with a polymerase such as *Taq* polymerase, and the polymerase amplifies the cDNA region between the two primers.

Another means of preparing a nucleic acid molecule encoding the amino acid sequence of an IL-17 like polypeptide, including a fragment or variant, is chemical synthesis using methods well known to the skilled artisan such as those described by Engels *et al.*, *Angew. Chem. Intl. Ed.*, 28:716-734 (1989). These methods include, *inter alia*, the

phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the amino acid sequence of an IL-17 like polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length nucleotide sequence of an IL-17 like polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the IL-17 like polypeptide, depending on whether the polypeptide produced in the host cell is designed to be secreted from that cell. Other methods known to the skilled artisan may be used as well.

In some cases, it may be desirable to prepare nucleic acid molecules encoding IL-17 like polypeptide variants. Nucleic acid molecules encoding variants may be produced using site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired point mutations (see Sambrook et al., supra, and Ausubel et al., supra, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels et al., supra, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have been altered for the optimal expression of an IL-17 like polypeptide in a given host cell. Particular codon alterations will depend upon the IL-17 like polypeptide(s) and host cell(s) selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host cell. Computer algorithms which incorporate codon frequency tables such as "Ecohigh.cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI. Other useful codon frequency tables include "Celegans_high.cod", "Celegans_low.cod", "Drosophila_high.cod", "Human_high.cod", "Maize_high.cod", and "Yeast_high.cod".

In other embodiments, nucleic acid molecules encode IL-17 like variants with conservative amino acid substitutions as described herein, IL-17 like variants comprising an

addition and/or a deletion of one or more N-linked or O-linked glycosylation sites, IL-17 like variants having deletions and/or substitutions of one or more cysteine residues, or IL-17 like polypeptide fragments as described herein. In addition, nucleic acid molecules may encode any combination of IL-17 like variants, fragments, and fusion polypeptides described herein.

Vectors and Host Cells

A nucleic acid molecule encoding the amino acid sequence of an IL-17 like polypeptide is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding the amino acid sequence of an IL-17 like polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems), and/or eukaryotic host cells. Selection of the host cell will depend in part on whether an IL-17 like polypeptide is to be post-translationally modified (e.g., glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian host cells are preferable. For a review of expression vectors, see Meth. Enz., v.185, D.V. Goeddel, ed. Academic Press Inc., San Diego, CA (1990).

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, *i.e.*, an oligonucleotide molecule located at the 5' or 3' end of the IL-17 like polypeptide coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or other "tag" such as FLAG, HA (hemaglutinin Influenza virus) or *myc* for which commercially available antibodies exist.

This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the IL-17 like polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified IL-17 like polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source) or synthetic, or the flanking sequences may be native sequences which normally function to regulate IL-17 like polypeptide expression. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequences are functional in, and can be activated by, the host cell machinery.

The flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein other than the endogenous IL-17 like gene flanking sequences will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with suitable oligonucleotide and/or flanking sequence fragments from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The

selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of an IL-17 like polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (Product No. 303-3s, New England Biolabs, Beverly, MA) is suitable for most Gram-negative bacteria and various origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV) or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of

a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the selection gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to the amplification of both the selection gene and the DNA that encodes an IL-17 like polypeptide. As a result, increased quantities of IL-17 like polypeptide are synthesized from the amplified DNA.

A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of an IL-17 like polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth herein and used in a prokaryotic vector.

A leader, or signal, sequence may be used to direct an IL-17 like polypeptide out of the host cell. Typically, a nucleotide sequence encoding the signal sequence is positioned in the coding region of an IL-17 like nucleic acid molecule, or directly at the 5' end of an IL-17 like polypeptide coding region. Many signal sequences have been identified, and any of those that are functional in the selected host cell may be used in conjunction with an IL-17 like nucleic acid molecule. Therefore, a signal sequence may be homologous (naturally occurring) or heterologous to an IL-17 like gene or cDNA. Additionally, a signal sequence may be chemically synthesized using methods described herein. In most cases, the secretion of an IL-17 like polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the secreted IL-17 like polypeptide. The signal sequence may be a component of the vector, or it may be a part of an IL-17 like nucleic acid molecule that is inserted into the vector.

Included within the scope of this invention is the use of either a nucleotide sequence encoding a native IL-17 like polypeptide signal sequence joined to an IL-17 like polypeptide coding region or a nucleotide sequence encoding a heterologous signal sequence joined to an

IL-17 like polypeptide coding region. The heterologous signal sequence selected should be one that is recognized and processed, i.e., cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native IL-17 like polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, the native IL-17 like polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add presequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the N-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired IL-17 like polypeptide, if the enzyme cuts at such area within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule is increased by the presence of one or more introns in the vector; this is particularly true where a polypeptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the IL-17 like gene, especially where the gene used is a full length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron(s) may be obtained from another source. The position of the intron with respect to flanking sequences and the IL-17 like gene is generally important, as the intron must be transcribed to be effective. Thus, when an IL-17 like cDNA molecule is being transcribed, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA transcription termination sequence. Preferably, the intron or introns will be located on one side or the other (i.e., 5' or 3') of the cDNA such that it does not interrupt the coding sequence. Any intron from any source, including any viral, prokaryotic and

eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell(s) into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

The expression and cloning vectors of the present invention will each typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding a IL-17 like polypeptide. Promoters are untranscribed sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription of the structural gene. Promoters are conventionally grouped into one of two classes, inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, initiate continual gene product production; that is, there is little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding an IL-17 like polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector. The native IL-17 like gene promoter sequence may be used to direct amplification and/or expression of an IL-17 like nucleic acid molecule. A heterologous promoter is preferred, however, if it permits greater transcription and higher yields of the expressed protein as compared to the native promoter, and if it is compatible with the host cell system that has been selected for use.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence(s), using linkers or adapters as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as

Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (CMV), a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, e.g., heat-shock promoters and the actin promoter.

Additional promoters which may be of interest in controlling IL-17 like gene transcription include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, Nature, 290:304-310, 1981); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797, 1980); the herpes thymidine kinase promoter (Wagner et al., Proc. Natl. Acad. Sci. USA, 78:144-1445, 1981); the regulatory sequences of the metallothionine gene (Brinster et al., Nature, 296:39-42, 1982); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff, et al., Proc. Natl. Acad. Sci. USA, 75:3727-3731, 1978); or the tac promoter (DeBoer, et al., Proc. Natl. Acad. Sci. USA, 80:21-25, 1983). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift et al., Cell, 38:639-646, 1984; Ornitz et al., Cold Spring Harbor Symp. Quant. Biol., 50:399-409 (1986); MacDonald, Hepatology, 7:425-515, 1987); the insulin gene control region which is active in pancreatic beta cells (Hanahan, Nature, 315:115-122, 1985); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., Cell, 38:647-658 (1984); Adames et al., Nature, 318:533-538 (1985); Alexander et al., Mol. Cell. Biol., 7:1436-1444, 1987); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., Cell, 45:485-495, 1986); the albumin gene control region which is active in liver (Pinkert et al., Genes and Devel., 1:268-276, 1987); the alphafetoprotein gene control region which is active in liver (Krumlauf et al., Mol. Cell. Biol., 5:1639-1648, 1985; Hammer et al., Science, 235:53-58, 1987); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., Genes and Devel., 1:161-171, 1987); the beta-globin gene control region which is active in myeloid cells (Mogram et al., Nature, 315:338-340, 1985; Kollias et al., Cell, 46:89-94, 1986); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., Cell, 48:703-712, 1987); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani, Nature, 314:283-286,

1985); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., Science, 234:1372-1378, 1986).

An enhancer sequence may be inserted into the vector to increase the transcription of a DNA encoding an IL-17 like polypeptide of the present invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent. They have been found 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to an IL-17 like nucleic acid molecule, it is typically located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the desired flanking sequences are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, Carlsbad, CA), pBSII (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids, plasmids or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO™ TA Cloning® Kit, PCR2.1® plasmid derivatives,

Invitrogen, Carlsbad, CA), and mammalian, yeast, or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). The recombinant molecules can be introduced into hose cells via transformation, transfection, infection. Electroporation, or other known techniques.

After the vector has been constructed and a nucleic acid molecule encoding an IL-17 like polypeptide has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for an IL-17 like polypeptide into a selected host cell may be accomplished by well known methods including methods such as transfection, infection, calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell or a vertebrate cell). The host cell, when cultured under appropriate conditions, synthesizes an IL-17 like polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. Examples include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR-cells (Urlaub et al., Proc. Natl. Acad. Sci. USA, 97:4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host

cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the ATCC. Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of E. coli (e.g., HB101, (ATCC No. 33694) DH5α, DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas spp., other Bacillus spp., Streptomyces spp., and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, Saccharomyces cerivisae and Pichia pastoris.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts et al., Biotechniques, 14:810-817 (1993); Lucklow, Curr. Opin. Biotechnol., 4:564-572 (1993); and Lucklow et al. (J. Virol., 67:4566-4579 (1993). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

One may also use transgenic animals to express glycosylated IL-17 like polypeptides. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide in the animal milk. One may also use plants to produce IL-17 like polypeptides, however, in general, the glycosylation occurring in plants is different from that produced in mammalian cells, and may result in a glycosylated product which is not suitable for human therapeutic use.

Polypeptide Production

Host cells comprising an IL-17 like polypeptide expression vector may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary to allow for the growth and survival of the cells. Suitable media for culturing E. coli cells include for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells include Roswell Park Memorial Institute medium 1640 (RPMI 1640), Minimal Essential Medium (MEM) and/or Dulbecco's Modified Eagle Medium (DMEM), all of which may be supplemented with serum and/or growth factors as indicated by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate and/or fetal calf serum, as necessary.

Typically, an antibiotic or other compound useful for selective growth of transformed cells is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline, and neomycin.

The amount of an IL-17 like polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, High Performance Liquid Chromatography (HPLC) separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If an IL-17 like polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. If however, the IL-17 like polypeptide is not secreted from the host cells, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells).

For an IL-17 like polypeptide situation in the host cell cytoplasm and/pr nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells), the host cells are typically disrupted mechanically or with a detergent to release the intracellular contents into a buffered solution. IL-17 like polypeptide can then be isolated from this solution.

If an IL-17 like polypeptide is produced intracellularly, the intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

If an IL-17 like polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with a chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. This solubilized IL-17 like polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate the IL-17 like polypeptide, isolation may be accomplished using standard methods such as those described herein and in Marston et al., Meth. Enz., 182:264-275 (1990).

In some cases, an IL-17 like polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/ dithiane DTT, and 2-2mercaptoethanol(bME)/dithio-b(ME). A cosolvent may be used to increase the efficiency of the refolding, and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

If inclusion bodies are not formed to a significant degree upon expression of an IL-17 like polypeptide, then the polypeptide will be found primarily in the supernatant after centrifugation of the cell homogenate. The polypeptide may be further isolated from the supernatant using methods such as those described herein.

The purification of an IL-17 like polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (IL-17 like polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or myc (Invitrogen, Carlsbad, CA) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of IL-17 like polypeptide/polyHis. See for example, Ausubel et al., eds., Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York (1993).

Additionally, the IL-17 like polypeptide may be purified through the use of a monoclonal antibody which is capable of specifically recognizing and binding to the IL-17 like polypeptide.

Suitable procedures for purification thus include, without limitation, affinity chromatography, immunoaffinity chromatography, ion exchange chromatography, molecular sieve chromatography, High Performance Liquid Chromatography (HPLC), electrophoresis (including native gel electrophoresis) followed by gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific, San Francisco, CA). In some cases, two or more purification techniques may be combined to achieve increased purity.

IL-17 like polypeptides, including fragments, variants, and/or derivatives thereof may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art, such as those set forth by Merrifield et al., J. Am. Chem. Soc., 85:2149 (1963), Houghten et al., Proc Natl Acad. Sci. USA, 82:5132 (1985), and Stewart and Young, Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL (1984). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized IL-17 like polypeptides may be oxidized using methods set forth in

these references to form disulfide bridges. Chemically synthesized IL-17 like polypeptides are expected to have comparable biological activity to the corresponding IL-17 like polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with a recombinant or natural IL-17 like polypeptide.

Another means of obtaining an IL-17 like polypeptide is via purification from biological samples such as source tissues and/or fluids in which the IL-17 like polypeptide is naturally found. Such purification can be conducted using methods for protein purification as described herein. The presence of the IL-17 like polypeptide during purification may be monitored using, for example, an antibody prepared against recombinantly produced IL-17 like polypeptide or peptide fragments thereof.

A number of additional methods for producing nucleic acids and polypeptides are known in the art, and the methods can be used to produce polypeptides having specificity for IL-17 like. See for example, Roberts et al., Proc. Natl. Acad. Sci. USA, 94:12297-12303 (1997), which describes the production of fusion proteins between an mRNA and its encoded peptide. See also Roberts, R., Curr. Opin. Chem. Biol., 3:268-273 (1999). Additionally, U.S. Patent No. 5,824,469 describes methods of obtaining oligonucleotides capable of carrying out a specific biological function. The procedure involves generating a heterogeneous pool of oligonucleotides, each having a 5' randomized sequence, a central preselected sequence, and a 3' randomized sequence. The resulting heterogeneous pool is introduced into a population of cells that do not exhibit the desired biological function. Subpopulations of the cells are then screened for those which exhibit a predetermined biological function. From that subpopulation, oligonucleotides capable of carrying out the desired biological function are isolated.

U.S. Patent Nos. 5,763,192, 5,814,476, 5,723,323, and 5,817,483 describe processes for producing peptides or polypeptides. This is done by producing stochastic genes or fragments thereof, and then introducing these genes into host cells which produce one or more proteins encoded by the stochastic genes. The host cells are then screened to identify those clones producing peptides or polypeptides having the desired activity.

Another method for producing peptides or polypeptides is described in PCT/US98/20094 (WO99/15650) filed by Athersys, Inc. Known as "Random Activation of Gene Expression for Gene Discovery" (RAGE-GD), the process involves the activation of

endogenous gene expression or over-expression of a gene by in situ recombination methods. For example, expression of an endogenous gene is activated or increased by integrating a regulatory sequence into the target cell which is capable of activating expression of the gene by non-homologous or illegitimate recombination. The target DNA is first subjected to radiation, and a genetic promoter inserted. The promoter eventually locates a break at the front of a gene, initiating transcription of the gene. This results in expression of the desired peptide or polypeptide.

It will be appreciated that these methods can also be used to create comprehensive IL-17 like protein expression libraries, which can subsequently be used for high throughput phenotypic screening in a variety of assays, such as biochemical assays, cellular assays, and whole organism assays (e.g., plant, mouse, etc.).

Chemical Derivatives

Chemically modified derivatives of the IL-17 like polypeptides may be prepared by one skilled in the art, given the disclosures set forth hereinbelow. IL-17 like polypeptide derivatives are modified in a manner that is different, either in the type or location of the molecules naturally attached to the polypeptide. Derivatives may include molecules formed by the deletion of one or more naturally-attached chemical groups. The polypeptide comprising the amino acid sequence of SEQ ID NO. 2, or an IL-17 like polypeptide variant may be modified by the covalent attachment of one or more polymers. For example, the polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Included within the scope of suitable polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

The polymers each may be of any molecular weight and may be branched or unbranched. The polymers each typically have an average molecular weight of between about 2kDa to about 100kDa (the term "about" indicating that in preparations of a water soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer preferably is between about 5kDa and about 50kDa, more preferably between about 12kDa and about 40kDa and most preferably between about 20kDa and about 35kDa.

Suitable water soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, carbohydrates, sugars,phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C_1-C_{10}) alkoxy- or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran, of, for example about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol) and polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules which may be used to prepare covalently attached multimers of the polypeptide comprising the amino acid sequence of SEQ ID NO. 2 or an IL-17 like polypeptide variant.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemical derivatives of polypeptides will generally comprise the steps of (a) reacting the polypeptide with the activated polymer molecule (such as a reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby the polypeptide comprising the amino acid sequence of SEQ ID NO. 2, or an IL-17 like polypeptide variant becomes attached to one or more polymer molecules, and (b) obtaining the reaction product(s). The optimal reaction conditions will be determined based on known parameters and the desired result. For example, the larger the ratio of polymer molecules:protein, the greater the percentage of attached polymer molecule. In one embodiment, the IL-17 like polypeptide derivative may have a single polymer molecule moiety at the amino terminus. See, for example, U.S. Patent No. 5,234,784.

The pegylation of the Il-17 like polypeptides may be specifically carried out by any of the pegylation reactions known in the art, as described for example in the following references: Francis *et al.*, *Focus on Growth Factors*, 3:4-10 (1992); EP 0154316; EP 0401384 and U.S. Patent No. 4,179,337. For example, pegylation may be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, the polymer(s) selected should have a single reactive ester group. For reductive

alkylation, the polymer(s) selected should have a single reactive aldehyde group. A reactive aldehyde is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Patent No. 5,252,714).

In another embodiment, IL-17 like polypeptides may be chemically coupled to biotin, and the biotin/IL-17 like polypeptide molecules which are conjugated are then allowed to bind to avidin, resulting in tetravalent avidin/biotin/IL-17 like polypeptide molecules. IL-17 like polypeptides may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates with a valency of 10.

Generally, conditions which may be alleviated or modulated by the administration of the present IL-17 like polypeptide derivatives include those described herein for IL-17 like polypeptides. However, the IL-17 like polypeptide derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to the non-derivatized molecules.

Microarray.

It will be appreciated that DNA microarray technology can be utilized in accordance with the present invention. DNA microarrays are miniature, high density arrays of nucleic acids positioned on a solid support, such as glass. Each cell or element within the array has numerous copies of a single species of DNA which acts as a target for hybridization for its cognate mRNA. In expression profiling using DNA microarray technology, mRNA is first extracted from a cell or tissue sample and then converted enzymatically to fluorescently labeled cDNA. This material is hybridized to the microarray and unbound cDNA is removed by washing. The expression of discrete genes represented on the array is then visualized by quantitating the amount of labeled cDNA which is specifically bound to each target DNA. In this way, the expression of thousands of genes can be quantitated in a high throughput, parallel manner from a single sample of biological material.

This high throughput expression profiling has a broad range of applications with respect to the TNFr/OGP-like molecules of the invention, including, but not limited to: the identification and validation of TNFr/OGP-like disease-related genes as targets for

therapeutics; molecular toxicology of TNFr/OGP-like molecules and inhibitors thereof; stratification of populations and generation of surrogate markers for clinical trials; and the enhancement of TNFr/OGP-like related small molecule drug discovery by aiding in the identification of selective compounds in high throughput screens (HTS).

5

Genetically Engineered Non-Human Animals

Additionally included within the scope of the present invention are non-human animals such as mice, rats, or other rodents, rabbits, goats, or sheep, or other farm animals, in which the gene (or genes) encoding the native IL-17 like polypeptide has (have) been disrupted ("knocked out") such that the level of expression of this gene or genes is (are) significantly decreased or completely abolished. Such animals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032.

The present invention further includes non-human animals such as mice, rats, or other rodents, rabbits, goats, sheep, or other farm animals, in which either the native form of the IL-17 like gene(s) for that animal or a heterologous IL-17 like gene(s) is (are) over-expressed by the animal, thereby creating a "transgenic" animal. Such transgenic animals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT application No. WO94/28122.

The present invention further includes non-human animals in which the promoter for one or more of the IL-17 like polypeptides of the present invention is either activated or inactivated (e.g., by using homologous recombination methods) to alter the level of expression of one or more of the native IL-17 like polypeptides.

These non-human animals may be used for drug candidate screening. In such screening, the impact of a drug candidate on the animal may be measured. For example, drug candidates may decrease or increase the expression of the IL-17 like gene. In certain embodiments, the amount of IL-17 like polypeptide, that is produced may be measured after the exposure of the animal to the drug candidate. Additionally, in certain embodiments, one may detect the actual impact of the drug candidate on the animal. For example, the overexpression of a particular gene may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease expression of the gene or its ability to prevent or inhibit a pathological condition. In other

examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product or its ability to prevent or inhibit a pathological condition.

5

Selective Binding Agents

As used herein, the term "selective binding agent" refers to a molecule which has specificity for one or more IL-17 like polypeptides. Suitable selective binding agents include, but are not limited to, antibodies and derivatives thereof, polypeptides, and small molecules. 10 Suitable selective binding agents may be prepared using methods known in the art. An exemplary IL-17 like polypeptide selective binding agent of the present invention is capable of binding a certain portion of the IL-17 like polypeptide thereby inhibiting the binding of the polypeptide to the IL-17 like polypeptide receptor(s).

Selective binding agents such as antibodies and antibody fragments that bind IL-17 15 like polypeptides are within the scope of the present invention. The antibodies may be polyclonal including monospecific polyclonal, monoclonal (MAbs), recombinant, chimeric, humanized such as CDR-grafted, human, single chain, and/or bispecific, as well as fragments, variants or derivatives thereof. Antibody fragments include those portions of the antibody which bind to an epitope on the IL-17 like polypeptide. Examples of such 20 fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies directed toward an IL-17 like polypeptide generally are 25 produced in animals (e.g., rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of IL-17 like polypeptide, and an adjuvant. It may be useful to conjugate an IL-17 like polypeptide or a variant, fragment, or derivative to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents

such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-IL-17 like polypeptide antibody titer.

Monoclonal antibodies directed toward an IL-17 like polypeptide are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler et al., Nature, 256:495-497 (1975) and the human B-cell hybridoma method, Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987). Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with IL-17 like polypeptides.

Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity. See, U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci., 81:6851-6855 (1985).

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed, for example, using methods known in the art. (See U.S. patent nos. 5,585,089 and 5,693,762). Generally, a humanized antibody has one or more amino acid residues introduced into if from a source which is non-human. Humanization can be performed, for example, using methods known in the art(Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science 239:1534-1536 (1988)), by substituting at least a portion of a rodent complementarity-determining regions (CDRs) for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies which bind IL-17 like polypeptides. Using transgenic animals (e.g., mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production, such antibodies are produced by immunization with an IL-17 like antigen (i.e., having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, for example, Jakobovits et al., Proc. Natl. Acad. Sci., 90:2551-2555 (1993); Jakobovits et al., Nature 362:255-258 (1993); Bruggermann et al., Year in Immuno., 7:33 (1993). In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, that is those having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human variable regions, including human (rather than e.g., murine) amino acid sequences, including variable regions, including human which are immunospecific for these antigens. See PCT application nos. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT application nos. PCT/US91/245, PCT/GB89/01207, and in EP 546073B1 and EP 546073A1. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In an alternative embodiment, human antibodies can be produced from phage-display libraries (Hoogenboom et al., J. Mol. Biol. 227:381 (1991); Marks et al., J. Mol. Biol. 222:581 (1991). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Application no. PCT/US98/17364, which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk- receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (e.g.,

human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-IL-17 like antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Sola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987)) for the detection and quantitation of IL-17 like polypeptides. The antibodies will bind IL-17 like polypeptides with an affinity which is appropriate for the assay method being employed.

For diagnostic applications, in certain embodiments, anti-IL-17 like antibodies may be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as 3H, 14C, 32P, 35S, or 125I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, -galactosidase, or horseradish peroxidase (Bayer et al., Meth. Enz., 184:138-163 (1990)).

Competitive binding assays rely on the ability of a labeled standard (e.g., an IL-17 like polypeptide, or an immunologically reactive portion thereof) to compete with the test sample analyte (an IL-17 like polypeptide) for binding with a limited amount of anti IL-17 like antibody. The amount of an IL-17 like polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex. See, *e.g.*, U.S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays)

or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assays). For example, one type of sandwich assay is an enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The selective binding agents, including anti-IL-17 like antibodies, also are useful for in vivo imaging. An antibody labeled with a detectable moiety may be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. The antibody may be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

The invention also relates to a kit comprising IL-17 like selective binding agents (such as antibodies) and other reagents useful for detecting IL-17 like polypeptide levels in biological samples. Such reagents may include a secondary activity, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

Selective binding agents of the invention, including antibodies, may be used as therapeutics. These therapeutic agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of an IL-17 like polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to an IL-17 like polypeptide and which are capable of inhibiting or eliminating the functional activity of an IL-17 like polypeptide in vivo or in vitro. In preferred embodiments, the selective binding agent (e.g., an antagonist antibody) will inhibit the functional activity of an IL-17 like polypeptide by at least about 50%, and preferably by at least about 80%. In another embodiment, the selective binding agent may be an anti-IL-17 polypeptide receptor antibody that is capable of interacting with an IL-17 like binding partner (a ligand or receptor) thereby inhibiting or eliminating IL-17 like activity in vitro or in vivo. Selective binding agents, including agonist and antagonist anti-IL-17 like antibodies, are identified by screening assays which are well known in the art.

IL-17 like polypeptides may be used to prepare IL-17 like polypeptide selective binding agents using methods known in the art. For example, an antigen may be used in a specific binding reaction to react, in a highly selective manner, with its corresponding

antibody(ies) and not with the multitude of other antibodies which can be evoked by other antigens.

The IL-17 like polypeptides of the present invention can be used to clone IL-17 like receptors, using an expression cloning strategy. Radiolabeled (125-Iodine) IL-17 like polypeptide or affinity/activity-tagged IL-17 like polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or cell line or tissue that expresses IL-17 like receptor(s). RNA isolated from such cells or tissues can be converted to cDNA, cloned into a mammalian expression vector, and transfected into mammalian cells (such as COS or 293 cells) to create an expression library. A radiolabeled or tagged IL-17 like polypeptide can then be used as an affinity ligand to identify and isolate from this library the subset of cells which express the IL-17 like receptor(s) on their surface. DNA can then be isolated from these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing IL-17 like receptor(s) is many-fold higher than in the original library. This enrichment process can be repeated iteratively until a single recombinant clone containing an IL-17 like receptor is isolated. Isolation of the IL-17 like receptor(s) is useful for identifying or developing novel agonists and antagonists of the IL-17 like polypeptide signaling pathway. Such agonists and antagonists include soluble IL-17 like receptor(s), anti-IL-17 like antibodies and/or anti-IL-17 like receptor antibodies, small molecules, or antisense oligonucleotides, and they may be used for treating one or more of the diseases/disorders described herein.

Additional Agonist and Antagonist Molecules

As defined herein, agonist or antagonist molecules either enhance or reduce, respectively, at least one of the biological activities of a IL-17 like polypeptide. Antagonists are capable of interacting with the IL-17 like receptor itself and/or with a IL-17 like binding partner (such as a ligand or receptor), thereby inhibiting or eliminating IL-17 like polypeptide activity in vitro or in vivo. Agonists are those molecules that can specifically bind to the IL-17 like molecule and function like their native ligands to activate the receptor. Agonists can also interact with a IL-17 like binding partner (such as a ligand) to enhance its binding to the IL-17 like polypeptides, thereby enhancing the biological activity of the IL-17 like molecule. It will be appreciated that the agonists and antagonists described herein are not limited to

selective binding agents. In addition to selective binding agents, other suitable agonist and antagonist molecules include, but are not limited to, soluble IL-17 like polypeptides, small molecules, and antisense oligonucleotides, any of which can be used for treating one or more disease or disorder, including those described herein.

IL-17 like polypeptides can be used to clone IL-17 like ligand(s) using an "expression cloning" strategy. Radiolabeled (125-Iodine) IL-17 like polypeptide or "affinity/activity-tagged" IL-17 like polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or a cell line or tissue that expresses IL-17 like ligand(s). RNA isolated from such cells or tissues can then be converted to cDNA, cloned into a mammalian expression vector, and transfected into mammalian cells (for example, COS, or 293) to create an expression library. Radiolabeled or tagged IL-17 like polypeptide can then be used as an affinity reagent to identify and isolate the subset of cells in this library expressing IL-17 like ligand(s). DNA is then isolated from these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing IL-17 like ligand(s) would be many-fold higher than in the original library. This enrichment process can be repeated iteratively until a single recombinant clone containing a IL-17 like ligand is isolated. Isolation of IL-17 like ligand(s) is useful for identifying or developing novel agonists and antagonists of the IL-17 like signaling pathway. Such agonists and antagonists include IL-17 like ligand(s), anti-IL-17 like ligand antibodies, small molecules or antisense oligonucleotides.

Assaying for other modulators of IL-17 like polypeptide activity

In some situations, it may be desirable to identify molecules that are modulators, i.e., agonists or antagonists, of the activity of IL-17 like polypeptide. Natural or synthetic molecules that modulate IL-17 like polypeptide may be identified using one or more screening assays, such as those described herein. Such molecules may be administered either in an ex vivo manner, or in an in vivo manner by injection, or by oral delivery, implantation device, or the like.

"Test molecule(s)" refers to the molecule(s) that is/are under evaluation for the ability to modulate (i.e., increase or decrease) the activity of an IL-17 like polypeptide. Most

commonly, a test molecule will interact directly with an IL-17 like polypeptide. However, it is also contemplated that a test molecule may also modulate IL-17 like polypeptide activity indirectly, such as by affecting IL-17 like gene expression, or by binding to an IL-17 like binding partner (e.g., receptor or ligand). In one embodiment, a test molecule will bind to an IL-17 like polypeptide with an affinity constant of at least about 10-6 M, preferably about 10-8 M, more preferably about 10-9 M, and even more preferably about 10-10 M.

Methods for identifying compounds which interact with IL-17 like polypeptides are encompassed by the present invention. In certain embodiments, an IL-17 like polypeptide is incubated with a test molecule under conditions which permit the interaction of the test molecule with an IL-17 like polypeptide, and the extent of the interaction can be measured. The test molecule(s) can be screened in a substantially purified form or in a crude mixture. The test molecules can be nucleic acid molecules, proteins, peptides, carbohydrates, lipids, organic and inorganic compounds.

In certain embodiments, an IL-17 like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with IL-17 like polypeptide to regulate its activity. Molecules which regulate IL-17 like polypeptide expression include nucleic acids which are complementary to nucleic acids encoding an IL-17 like polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of IL-17 like polypeptide, and which act as anti-sense regulators of expression.

Once a set of test molecules has been identified as interacting with an IL-17 like polypeptide, the molecules may be further evaluated for their ability to increase or decrease IL-17 like polypeptide activity. The measurement of the interaction of test molecules with IL-17 like polypeptides may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. In general, test molecules are incubated with an IL-17 like polypeptide for a specified period of time, and IL-17 like polypeptide activity is determined by one or more assays described herein for measuring biological activity.

The interaction of test molecules with IL-17 like polypeptides may also be assayed directly using polyclonal or monoclonal antibodies in an immunoassay. Alternatively,

modified forms of IL-17 like polypeptides containing epitope tags as described herein may be used in solution and immunoassays.

In certain embodiments, a IL-17 like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with IL-17 like polypeptide to regulate its activity. Potential protein antagonists of IL-17 like polypeptide include antibodies which interact with active regions of the polypeptide and inhibit or eliminate at least one activity of IL-17 like molecules. Molecules which regulate IL-17 like polypeptide expression include nucleic acids which are complementary to nucleic acids encoding a IL-17 like polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of IL-17 like polypeptide, and which act as anti-sense regulators of expression.

In the event that IL-17 like polypeptides display biological activity through an interaction with a binding partner (e.g., a selective binding agent, receptor, or ligand), a variety of in vitro assays may be used to measure the binding of an IL-17 like polypeptide to the corresponding binding partner such as a selective binding agent or ligand. These assays may be used to screen test molecules for their ability to increase or decrease the rate and/or the extent of binding of an IL-17 like polypeptide to its binding partner. In one assay, an IL-17 like polypeptide is immobilized in the wells of a microtiter plate. Radiolabeled IL-17 like binding partner (for example, iodinated IL-17 like binding partner) and the test molecule(s) can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can be washed and counted, using a scintillation counter, for radioactivity to determine the extent to which the binding partner bound to IL-17 like polypeptide. Typically, the molecules will be tested over a range of concentrations, and a series of control wells lacking one or more elements of the test assays can be used for accuracy in the evaluation of the results. An alternative to this method involves reversing the "positions" of thepolypeptides, i.e., immobilizing IL-17 like binding partner to the microtiter plate wells, incubating with the test molecule and radiolabeled IL-17 like polypeptide, and determining the extent of IL-17 like polypeptide binding. See, for example, chapter 18, Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, New York, NY (1995).

As an alternative to radiolabelling, an IL-17 like polypeptide or its binding partner may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), that can be detected colorometrically, or by fluorescent tagging of streptavidin. An antibody directed to an IL-17 like polypeptide or to an IL-17 like binding partner and conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP.

An IL-17 like polypeptide or an IL-17 like binding partner can also be immobilized by attachment to agarose beads, acrylic beads or other types of such inert solid phase substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound. After incubation, the beads can be precipitated by centrifugation, and the amount of binding between an IL-17 like polypeptide and its binding partner can be assessed using the methods described herein. Alternatively, the substrate-protein complex can be immobilized in a column, and the test molecule and complementary protein are passed through the column. The formation of a complex between an IL-17 like polypeptide and its binding partner can then be assessed using any of the techniques set forth herein, i.e., radiolabelling, antibody binding, or the like.

Another in vitro assay that is useful for identifying a test molecule which increases or decreases the formation of a complex between an IL-17 like binding protein and an IL-17 like binding partner is a surface plasmon resonance detector system such as the BIAcore assay system (Pharmacia, Piscataway, NJ). The BIAcore system may be carried out using the manufacturer's protocol. This assay essentially involves the covalent binding of either IL-17 like polypeptide or an IL-17 like binding partner to a dextran-coated sensor chip which is located in a detector. The test compound and the other complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test compounds together for their ability to increase or decrease the formation of a complex between an IL-17 like

polypeptide and an IL-17 like binding partner. In these cases, the assays set forth herein can be readily modified by adding such additional test compound(s) either simultaneous with, or subsequent to, the first test compound. The remainder of the steps in the assay are as set forth herein.

In vitro assays such as those described herein may be used advantageously to screen large numbers of compounds for effects on complex formation by IL-17 like polypeptide and IL-17 like binding partner. The assays may be automated to screen compounds generated in phage display, synthetic peptide, and chemical synthesis libraries.

Compounds which increase or decrease the formation of a complex between an IL-17 like polypeptide and an IL-17 like binding partner may also be screened in cell culture using cells and cell lines expressing either IL-17 like polypeptide or IL-17 like binding partner. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of an IL-17 like polypeptide to cells expressing IL-17 like binding partner at the surface is evaluated in the presence or absence of test molecules, and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to an IL-17 like binding partner. Cell culture assays can be used advantageously to further evaluate compounds that score positive in protein binding assays described herein.

Cell cultures can also be used to screen the impact of a drug candidate. For example, drug candidates may decrease or increase the expression of the IL-17 like gene. In certain embodiments, the amount of IL-17 like polypeptide or a fragment(s) that is produced may be measured after exposure of the cell culture to the drug candidate. In certain embodiments, one may detect the actual impact of the drug candidate on the cell culture. For example, the overexpression of a particular gene may have a particular impact on the cell culture. In such cases, one may test a drug candidate's ability to increase or decrease the expression of the gene or its ability to prevent or inhibit a particular impact on the cell culture. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product in a cell culture.

A yeast two-hybrid system (Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-9583, 1991) can be used to identify novel polypeptides that bind to, or interact with, IL-17 like polypeptides. As an example, a yeast-two hybrid bait construct can be generated in a vector (such as the pAS2-1 from Clontech) which encodes a yeast GAL4-DNA binding domain fused to the IL-17 like polynucleotide. This bait construct may be used to screen human cDNA libraries wherein the cDNA library sequences are fused to GAL4 activation domains. Positive interactions will result in the activation of a reporter gene such as β-Gal. Positive clones emerging from the screening may be characterized further to identify interacting proteins.

Internalizing Proteins

The TAT protein sequence (from HIV) can be used to internalize proteins into a cell by targeting the lipid bi-layer component of the cell membrane. See e.g., Falwell et al., Proc. Natl. Acad. Sci., 91: 664-668, 1994. For example, an 11 amino acid sequence (YGRKKRRQRRR; SEQ ID NO: 11) of the HIV TAT protein (termed the "protein transduction domain", or TAT PDT) has been shown to mediate delivery of large bioactive proteins such as β-galactosidase and p27Kip across the cytoplasmic membrane and the nuclear membrane of a cell. See Schwarze et al., Science, 285: 1569-1572, 1999; and Nagahara et al., Nature Medicine, 4: 1449-1452, 1998. Schwartze et al. (Science, 285: 1569-72, 1999) demonstrated that cultured cells acquired β-gal activity when exposed to a fusion of the TAT PDT and β-galactosidase. Injection of mice with the TAT-β-gal fusion proteins resulted in β-gal expression in a number of tissues, including liver, kidney, lung, heart, and brain tissue.

It will thus be appreciated that the TAT protein sequence may be used to internalize a desired protein or polypeptide into a cell. In the context of the present invention, the TAT protein sequence can be fused to another molecule such as a IL-17-like antagonist (i.e.: anti-Il-17-like selective binding agent or small molecule) and administered intracellularly to inhibit the activity of the IL-17-like molecule. Where desired, the IL-17-like protein itself, or a peptide fragment or modified form of IL-17-like, may be fused to such a protein transducer for administrating to cells using the procedures, described above.

Therapeutic Uses

A non-exclusive list of uses and treatments for the IL-17 like antagonists of the invention includes: the treatment or prevention of inflammatory disease, autoimmune disease, allergies, asthma, and organ or graft rejection in a patient. The IL-17 antagonists of the invention are also useful for inhibiting T cell proliferation and/or activation, for inhibiting in vivo B cell proliferation or immunoglobulin secretion, and for blocking the effects of IL-17 in inducing bone destruction.

As contemplated by the present invention, an IL-17 like polypeptide, agonist or antagonist thereof may be administered as an adjunct to other therapy and also with other pharmaceutical agents suitable for the indication being treated. An IL-17 like polypeptide and any of one or more additional therapies or pharmaceutical agents may be administered separately, sequentially, or simultaneously.

In a specific embodiment, the present invention is also directed to the use of an IL-17 like polypeptide or an antagonist of the IL-17 like molecule in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more interleukin-1 (IL-1) inhibitors for the treatment of diseases treatable with IL-17 like polypeptide or an antagonist of the IL-17 like molecule. Classes of interleukin-1 inhibitors include interleukin-1 receptor antagonists (any compound capable of specifically preventing activation of cellular receptors to IL-1) such as IL-1ra, as described below; anti-IL-1 receptor monoclonal antibodies (e.g., EP 623674, the disclosure of which is hereby incorporated by reference; IL-1 binding proteins such as soluble IL-1 receptors (e.g., U.S. Patent Nos. 5,492,888, 5,488,032, 5,464,937, 5,319,071 and 5,180,812, the disclosure of which are hereby incorporated by reference); anti-IL-1 monoclonal antibodies (e.g., WO 95/01997, WO 94/02627, WO 90/06371, U.S. Patent No. 4,935,343, EP 364778, EP 267611 and EP 220063); IL-1 receptor accessory proteins (e.g., WO 96/23067), and other compounds and proteins which block in vivo synthesis or extracellular release of IL-1.

Interleukin-1 receptor antagonist (IL-1ra) is a human protein that acts as a natural inhibitor of interleukin-1. Interleukin-1 receptor antagonists, as well as the methods of making and methods of using thereof, are described in U.S. Patent No. 5,075,222;

WO 91/08285; WO 91/17184; AU 9173636; WO 92/16221; WO 93/21946; WO 94/06457; WO 94/21275; FR 2706772; WO 94/21235; DE 4219626; WO 94/20517; WO 96/22793 and WO 97/28828, the disclosure of which are hereby incorporated by reference. The proteins include glycosylated as well as non-glycosylated IL-1 receptor antagonists.

Specifically, three exemplary forms of IL-1ra (IL-1raα, IL-1raβ and IL-1rax), are disclosed and described in U.S. Patent No. 5,075,222. Methods for producing IL-1 inhibitors, particularly IL-1ras, are also disclosed in the 5,075,222 patent.

An additional class of interleukin-1 inhibitors includes compounds capable of specifically preventing activation of cellular receptors to IL-1. Such compounds include IL-1 binding proteins, such as soluble receptors and monoclonal antibodies. Such compounds also include monoclonal antibodies to the receptors.

A further class of interleukin-1 inhibitors includes compounds and proteins which block in vivo synthesis and/or extracellular release of IL-1. Such compounds include agents which affect transcription of IL-1 genes or processing of IL-1 preproteins.

In another embodiment, the present invention is directed to the use of an IL-17 like polypeptide or an antagonist of the IL-17 like molecule in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more TNF inhibitors for the treatment or prevention of the diseases and disorders recited herein.

Such TNF inhibitors include compounds and proteins which block in vivo synthesis or extracellular release of TNF. In a specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more of the following TNF inhibitors: TNF binding proteins (soluble TNF receptor type-I and soluble TNF receptor type-II ("sTNFRs"), as defined herein), anti-TNF antibodies, granulocyte colony stimulating factor; thalidomide; BN 50730; tenidap; E 5531; tiapafant PCA 4248; nimesulide; panavir; rolipram; RP 73401; peptide T; MDL 201,449A; (1R,3S)-Cis-1-[9-(2,6-diaminopurinyl)]-3-hydroxy-4-cyclopentene hydrochloride; (1R,3R)-trans-1-(9-(2,6-diamino)purine]-3-acetoxycyclopentane; (1R,3R)-trans-1-[9-adenyl)-3-azidocyclopentane hydrochloride and (1R,3R)-trans-1-(6-hydroxy-purin-9-yl)-3-azidocyclo-pentane. TNF binding proteins are disclosed in the art (EP 308 378, EP 422 339, GB 2 218 101, EP 393 438, WO 90/13575, EP 398 327, EP 412 486, WO 91/03553, EP 418 014, JP 127,800/1991, EP 433 900, U.S. Patent

No. 5,136,021, GB 2 246 569, EP 464 533, WO 92/01002, WO 92/13095, WO 92/16221, EP 512 528, EP 526 905, WO 93/07863, EP 568 928, WO 93/21946, WO 93/19777, EP 417 563, WO 94/06476, and PCT International Application No. PCT/US97/12244).

For example, EP 393 438 and EP 422 339 teach the amino acid and nucleic acid sequences of a soluble TNF receptor type I (also known as "sTNFR-I" or "30kDa TNF inhibitor") and a soluble TNF receptor type II (also known as "sTNFR-II" or "40kDa TNF inhibitor"), collectively termed "sTNFRs", as well as modified forms thereof (e.g., fragments, functional derivatives and variants). EP 393 438 and EP 422 339 also disclose methods for isolating the genes responsible for coding the inhibitors, cloning the gene in suitable vectors and cell types and expressing the gene to produce the inhibitors. Additionally, polyvalent forms (i.e., molecules comprising more than one active moiety) of sTNFR-I and sTNFR-II have also been disclosed. In one embodiment, the polyvalent form may be constructed by chemically coupling at least one TNF inhibitor and another moiety with any clinically acceptable linker, for example polyethylene glycol (WO 92/16221 and WO 95/34326), by a peptide linker (Neve et al. (1996), Cytokine, 8(5):365-370, by chemically coupling to biotin and then binding to avidin (WO 91/03553) and, finally, by combining chimeric antibody molecules (U.S. Patent 5,116,964, WO 89/09622, WO 91/16437 and EP 315062.

Anti-TNF antibodies include the MAK 195F Fab antibody (Holler et al. (1993), 1st International Symposium on Cytokines in Bone Marrow Transplantation, 147); CDP 571 anti-TNF monoclonal antibody (Rankin et al. (1995), British Journal of Rheumatology, 34:334-342); BAY X 1351 murine anti-tumor necrosis factor monoclonal antibody (Kieft et al. (1995), 7th European Congress of Clinical Microbiology and Infectious Diseases, page 9); CenTNF cA2 anti-TNF monoclonal antibody (Elliott et al. (1994), Lancet, 344:1125-1127 and Elliott et al. (1994), Lancet, 344:1105-1110).

In another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist thereof in combination (pretreatment, post-treatment, or concurrent treatment) with secreted or soluble human fas antigen or recombinant versions thereof (WO96/20206 and Mountz et al., J. Immunology, 155:4829-4837; and EP 510 691. WO96/20206 discloses secreted human fas antigen (native and recombinant, including an Ig fusion protein), methods for isolating the genes responsible for coding the soluble recombinant human fas antigen, methods for cloning the gene in suitable

vectors and cell types, and methods for expressing the gene to produce the inhibitors. EP 510 691 describes DNAs coding for human fas antigen, including soluble fas antigen, vectors expressing for said DNAs and transformants transfected with the vector. When administered parenterally, doses of a secreted or soluble fas antigen fusion protein each are generally from about 1 microgram/kg to about 100 micrograms/kg.

Treatment of the diseases and disorders recited herein can include the use of first line drugs for control of pain and inflammation. These drugs are classified as non-steroidal, anti-inflammatory drugs (NSAIDs). Secondary treatments include corticosteroids, slow acting antirheumatic drugs (SAARDs), or disease modifying (DM) drugs. Information regarding the following compounds can be found in The Merck Manual of Diagnosis and Therapy, Sixteenth Edition, Merck, Sharp & Dohme Research Laboratories, Merck & Co., Rahway, N.J. (1992) and in Pharmaprojects, PJB Publications Ltd.

In a specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist and any of one or more NSAIDs for the treatment of the diseases and disorders recited herein. NSAIDs owe their anti-inflammatory action, at least in part, to the inhibition of prostaglandin synthesis (Goodman and Gilman in "The Pharmacological Basis of Therapeutics," MacMillan 7th Edition (1985)). NSAIDs can be characterized into at least nine groups: (1) salicylic acid derivatives; (2) propionic acid derivatives; (3) acetic acid derivatives; (4) fenamic acid derivatives; (5) carboxylic acid derivatives; (6) butyric acid derivatives; (7) oxicams; (8) pyrazoles and (9) pyrazolones.

In another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more salicylic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. Such salicylic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: acetaminosalol, aloxiprin, aspirin, benorylate, bromosaligenin, calcium acetylsalicylate, choline magnesium trisalicylate, magnesium salicylate, choline salicylate, diflusinal, etersalate, fendosal, gentisic acid, glycol salicylate, imidazole salicylate, lysine acetylsalicylate, mesalamine, morpholine salicylate, 1-naphthyl salicylate, olsalazine, parsalmide, phenyl acetylsalicylate, phenyl salicylate, salacetamide, salicylamide O-acetic acid, salsalate, sodium salicylate and sulfasalazine.

Structurally related salicylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more propionic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The propionic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: alminoprofen, benoxaprofen, bucloxic acid, carprofen, dexindoprofen, fenoprofen, flunoxaprofen, fluprofen, flurbiprofen, furcloprofen, ibuprofen, ibuprofen aluminum, ibuproxam, indoprofen, isoprofen, ketoprofen, loxoprofen, miroprofen, naproxen, naproxen sodium, oxaprozin, piketoprofen, pimeprofen, pirprofen, pranoprofen, protizinic acid, pyridoxiprofen, suprofen, tiaprofenic acid and tioxaprofen. Structurally related propionic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In yet another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more acetic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The acetic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: acemetacin, alclofenac, amfenac, bufexamac, cinmetacin, clopirac, delmetacin, diclofenac potassium, diclofenac sodium, etodolac, felbinac, fenclofenac, fenclorac, fenclozic acid, fentiazac, furofenac, glucametacin, ibufenac, indomethacin, isofezolac, isoxepac, lonazolac, metiazinic acid, oxametacin, oxpinac, pimetacin, proglumetacin, sulindac, talmetacin, tiaramide, tiopinac, tolmetin, tolmetin sodium, zidometacin and zomepirac. Structurally related acetic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more fenamic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The fenamic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: enfenamic acid, etofenamate, flufenamic

acid, isonixin, meclofenamic acid, meclofenamate sodium, medofenamic acid, mefenamic acid, niflumic acid, talniflumate, terofenamate, tolfenamic acid and ufenamate. Structurally related fenamic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more carboxylic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The carboxylic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof which can be used comprise: clidanac, diflunisal, flufenisal, inoridine, ketorolac and tinoridine. Structurally related carboxylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In yet another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more butyric acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The butyric acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: bumadizon, butibufen, fenbufen and xenbucin. Structurally related butyric acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more oxicams, prodrug esters, or pharmaceutically acceptable salts thereof. The oxicams, prodrug esters, and pharmaceutically acceptable salts thereof comprise: droxicam, enolicam, isoxicam, piroxicam, sudoxicam, tenoxicam and 4-hydroxyl-1,2-benzothiazine 1,1-dioxide 4-(N-phenyl)-carboxamide. Structurally related oxicams having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more pyrazoles, prodrug esters, or pharmaceutically acceptable salts thereof. The pyrazoles, prodrug esters, and pharmaceutically acceptable salts

thereof which may be used comprise: difenamizole and epirizole. Structurally related pyrazoles having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment or, concurrent treatment) with any of one or more pyrazolones, prodrug esters, or pharmaceutically acceptable salts thereof. The pyrazolones, prodrug esters and pharmaceutically acceptable salts thereof which may be used comprise: apazone, azapropazone, benzpiperylon, feprazone, mofebutazone, morazone, oxyphenbutazone, phenylbutazone, pipebuzone, propylphenazone, ramifenazone, suxibuzone and thiazolinobutazone. Structurally related pyrazalones having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more of the following NSAIDs: ε-acetamidocaproic acid, S-adenosyl-methionine, 3-amino-4-hydroxybutyric acid, amixetrine, anitrazafen, antrafenine, bendazac, bendazac lysinate, benzydamine, beprozin, broperamole, bucolome, bufezolac, ciproquazone, cloximate, dazidamine, deboxamet, detomidine, difenpiramide, difenpyramide, difisalamine, ditazol, emorfazone, fanetizole mesylate, fenflumizole, floctafenine, flumizole, flunixin, fluproquazone, fopirtoline, fosfosal, guaimesal, guaiazolene, isonixim, lefetamine HCl, leflunomide, lofemizole, lotifazole, lysin clonixinate, meseclazone, nabumetone, nictindole, nimesulide, orgotein, orpanoxin, oxaceprol, oxapadol, paranyline, perisoxal, perisoxal citrate, pifoxime, piproxen, pirazolac, pirfenidone, proquazone, proxazole, thielavin B, tiflamizole, timegadine, tolectin, tolpadol, tryptamid and those designated by company code number such as 480156S, AA861, AD1590, AFP802, AFP860, AI77B, AP504, AU8001, BPPC, BW540C, CHINOIN 127, CN100, EB382, EL508, F1044, FK-506, GV3658, ITF182, KCNTEI6090, KME4, LA2851, MR714, MR897, MY309, ONO3144, PR823, PV102, PV108, R830, RS2131, SCR152, SH440, SIR133, SPAS510, SQ27239, ST281, SY6001, TA60, TAI-901 (4-benzoyl-1-indancarboxylic acid), TVX2706, U60257, UR2301 and WY41770. Structurally related

NSAIDs having similar analgesic and anti-inflammatory properties to the NSAIDs are also intended to be encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more corticosteroids, prodrug esters or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, graft versus host disease and multiple sclerosis. Corticosteroids, prodrug esters and pharmaceutically acceptable salts thereof include hydrocortisone and compounds which are derived from hydrocortisone, such as 21-acetoxypregnenolone, alclomerasone, algestone, amcinonide, beclomethasone, betamethasone, betamethasone valerate, budesonide, chloroprednisone, clobetasol, clobetasol propionate, clobetasone, clobetasone butyrate, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacon, desonide, desoximerasone, dexamethasone, diflorasone, diflucortolone, difluprednate, enoxolone, fluazacort, flucloronide, flumethasone, flumethasone pivalate, flucinolone acetonide, flunisolide, fluocinonide, fluorocinolone acetonide, fluocortin butyl, fluocortolone, fluocortolone hexanoate, diflucortolone valerate, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandenolide, formocortal, halcinonide, halometasone, halopredone acetate, hydro-cortamate, hydrocortisone, hydrocortisone acetate, hydro-cortisone butyrate, hydrocortisone phosphate, hydrocortisone 21-sodium succinate, hydrocortisone tebutate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 21-diedryaminoacetate, prednisolone sodium phosphate, prednisolone sodium succinate, prednisolone sodium 21-m-sulfobenzoate, prednisolone sodium 21-stearoglycolate, prednisolone tebutate, prednisolone 21-trimethylacetate, prednisone, prednival, prednylidene, prednylidene 21-diethylaminoacetate, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide and triamcinolone hexacetonide. Structurally related corticosteroids having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment,

or concurrent treatment) with any of one or more slow-acting antirheumatic drugs (SAARDs) or disease modifying antirheumatic drugs (DMARDS), prodrug esters, or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, graft versus host disease and multiple sclerosis. SAARDs or DMARDS, prodrug esters and pharmaceutically acceptable salts thereof comprise: allocupreide sodium, auranofin, aurothioglucose, aurothioglycanide, azathioprine, brequinar sodium, bucillamine, calcium 3-aurothio-2-propanol-1-sulfonate, chlorambucil, chloroquine, clobuzarit, cuproxoline, cyclophosphamide, cyclosporin, dapsone, 15-deoxyspergualin, diacerein, glucosamine, gold salts (e.g., cycloquine gold salt, gold sodium thiomalate, gold sodium thiosulfate), hydroxychloroquine, hydroxychloroquine sulfate, hydroxyurea, kebuzone, levamisole, lobenzarit, melittin, 6-mercaptopurine, methotrexate, mizoribine, mycophenolate mofetil, myoral, nitrogen mustard, D-penicillamine, pyridinol imidazoles such as SKNF86002 and SB203580, rapamycin, thiols, thymopoietin and vincristine. Structurally related SAARDs or DMARDs having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In yet embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more COX2 inhibitors, prodrug esters or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation. Examples of COX2 inhibitors, prodrug esters or pharmaceutically acceptable salts thereof include, for example, celecoxib. Structurally related COX2 inhibitors having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an IL-17 like polypeptide, agonist or antagonist in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more antimicrobials, prodrug esters or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation. Antimicrobials include, for example, the broad classes of penicillins, cephalosporins and other beta-lactams, aminoglycosides, azoles, quinolones, macrolides, rifamycins, tetracyclines, sulfonamides,

lincosamides and polymyxins. The penicillins include, but are not limited to penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, floxacillin, ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanate, hetacillin, cyclacillin, bacampicillin, carbenicillin, carbenicillin indanyl, ticarcillin, ticarcillin/clavulanate, azlocillin, mezlocillin, peperacillin, and mecillinam. The cephalosporins and other beta-lactams include, but are not limited to cephalothin, cephapirin, cephalexin, cephradine, cefazolin, cefadroxil, cefaclor, cefamandole, cefotetan, cefoxitin, ceruroxime, cefonicid, ceforadine, cefixime, cefotaxime, moxalactam, ceftizoxime, cetriaxone, cephoperazone, ceftazidime, imipenem and aztreonam. The aminoglycosides include, but are not limited to streptomycin, gentamicin, tobramycin, amikacin, netilmicin, kanamycin and neomycin. The azoles include, but are not limited to fluconazole. The quinolones include, but are not limited to nalidixic acid, norfloxacin, enoxacin, ciprofloxacin, ofloxacin, sparfloxacin and temafloxacin. The macrolides include, but are not limited to erythomycin, spiramycin and azithromycin. The rifamycins include, but are not limited to rifampin. The tetracyclines include, but are not limited to spicycline, chlortetracycline, clomocycline, demeclocycline, deoxycycline, guamecycline, lymecycline, meclocycline, methacycline, minocycline, oxytetracycline, penimepicycline, pipacycline, rolitetracycline, sancycline, senociclin and tetracycline. The sulfonamides include, but are not limited to sulfanilamide, sulfamethoxazole, sulfacetamide, sulfadiazine, sulfisoxazole and co-trimoxazole (trimethoprim/sulfamethoxazole). The lincosamides include, but are not limited to clindamycin and lincomycin. The polymyxins (polypeptides) include, but are not limited to polymyxin B and colistin.

IL-17 Like Compositions and Administration

Therapeutic compositions are within the scope of the present invention. Such IL-17 like pharmaceutical compositions may comprise a therapeutically effective amount of an IL-17 like polypeptide or an IL-17 like nucleic acid molecule in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Other pharmaceutical compositions may comprise a therapeutically effective amount of one or more IL-17 like selective binding agents in

admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Acceptable formulation materials preferably are nontoxic to recipients.

The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide), solvents (such as glycerin, propylene glycol or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal), stability enhancing agents (sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride), mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company [1990]).

The optimal pharmaceutical formulation will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See for example, Remington's Pharmaceutical Sciences, supra. Such

compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the IL-17 like molecule.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present invention, IL-17 like polypeptide compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, supra) in the form of a lyophilized cake or an aqueous solution. Further, the IL-17 like polypeptide product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The IL-17 like polypeptide pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired IL-17 like molecule in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a IL-17 like molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), or beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered as a depot injection. Hyaluronic acid may

also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

Pharmaceutical compositions such as (1) slow-release formulations, (2) inhalant mists, or (3) orally active formulations are also envisioned. The IL-17 like molecule pharmaceutical composition generally is formulated for parenteral administration. Such parenterally administered therapeutic compositions are typically in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired IL-17 like molecule in a pharmaceutically acceptable vehicle. The IL-17 like molecule pharmaceutical compositions also may include particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or the introduction of the molecule into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation.

In one embodiment, a pharmaceutical composition may be formulated for inhalation. For example, an IL-17 like polypeptide may be formulated as a dry powder for inhalation. IL-17 like polypeptide or IL-17 like nucleic acid molecule inhalation solutions may also be formulated with a liquefied propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT application no. PCT/US94/001875 which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, IL-17 like polypeptides which are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the IL-17 like polypeptide. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Another pharmaceutical composition may involve an effective quantity of IL-17 like polypeptides in a mixture with non-toxic excipients which are suitable for the manufacture of

tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional IL-17 like polypeptide formulations will be evident to those skilled in the art, including formulations involving IL-17 like polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 which describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer et al., J. Biomed. Mater. Res., 15:167-277 (1981) and Langer; Chem. Tech., 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., supra) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art. See e.g., Eppstein et al., Proc. Natl. Acad. Sci. USA, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

The IL-17 like pharmaceutical composition to be used for in vivo administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

An effective amount of an IL-17 like pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the IL-17 like molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 1 μg/kg up to about 100 mg/kg; or 5 μg/kg up to about 100 mg/kg; or 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg.

The frequency of dosing will depend upon the pharmacokinetic parameters of the IL-17 like molecule in the formulation used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, e.g. oral, inhalation, injection or infusion by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes, or by sustained release systems or implantation device. Where desired, the compositions may be administered continuously by infusion, by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation into the affected area of a membrane, sponge, or other appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be directly through the device via diffusion, time release bolus, or continuous administration, or via catheter using continuous infusion.

It will further be appreciated that the IL-17 like polypeptides, including fragments, variants, and derivatives, may be employed alone, together, or in combination with other polypeptides and pharmaceutical compositions. For example, the IL-17 like polypeptides may be used in combination with cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

In some cases, it may be desirable to use IL-17 like pharmaceutical compositions in an ex vivo manner. In such instances, cells, tissues, or organs that have been removed from the patient are exposed to IL-17 like pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, an IL-17 like polypeptide can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Additional embodiments of the present invention relate to cells and methods (e.g., homologous recombination and/or other recombinant production methods) for both the in vitro production of therapeutic polypeptides and for the production and delivery of therapeutic polypeptides by gene therapy or cell therapy.

It is further envisioned that IL-17 like polypeptide can be produced in vitro or in vivo by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding IL-17 like polypeptides. For example, homologous recombination methods may be used to modify a cell that contains a normally transcriptionally silent IL-17 like gene, or an under expressed gene, and thereby produce a cell which expresses therapeutically efficacious amounts of IL-17 like polypeptides.

Homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes. (Kucherlapati, Prog. in Nucl. Acid Res. & Mol. Biol., 36:301 (1989)). The basic technique was developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas et al., Cell, 44:419-428 (1986); Thomas and Capecchi, Cell, 51:503-512, 1987; Doetschman et al., Proc. Natl. Acad. Sci., 85:8583-8587, 1988) or to correct specific mutations within defective genes (Doetschman et al., Nature, 330:576-578, 1987). Exemplary homologous recombination techniques are described in U.S. Patent No. 5,272,071 (EP 9193051, EP Publication No. 505500; PCT/US90/07642, International Publication No. WO91/09955).

Through homologous recombination, the DNA sequence to be inserted into the genome can be directed to a specific region of the gene of interest by attaching it to targeting DNA. The targeting DNA is a nucleotide sequence that is complementary (homologous) to a region of the genomic DNA. Small pieces of targeting DNA that are complementary to a specific region of the genome are put in contact with the parental strand during the DNA replication process. It is a general property of DNA that has been inserted into a cell to hybridize, and therefore, recombine with other pieces of endogenous DNA through shared homologous regions. If this complementary strand is attached to an oligonucleotide that contains a mutation or a different sequence or an additional nucleotide, it too is incorporated into the newly synthesized strand as a result of the recombination. As a result of the

proofreading function, it is possible for the new sequence of DNA to serve as the template. Thus, the transferred DNA is incorporated into the genome.

Attached to these pieces of targeting DNA are regions of DNA which may interact with or control the expression of a IL-17 like polypeptide, e.g., flanking sequences. For example, a promoter/enhancer element, a suppresser, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired IL-17 like polypeptide. The control element controls a portion of the DNA present in the host cell genome. Thus, the expression of the desired IL-17 like polypeptide may be achieved not by transfection of DNA that encodes the IL-17 like gene itself, but rather by the use of targeting DNA (containing regions of homology with the endogenous gene of interest) coupled with DNA regulatory segments that provide the endogenous gene sequence with recognizable signals for transcription of an IL-17 like polypeptide.

In an exemplary method, the expression of a desired targeted gene in a cell (i.e., a desired endogenous cellular gene) is altered via homologous recombination into the cellular genome at a preselected site by the introduction of DNA which includes at least a regulatory sequence, an exon and a splice donor site. These components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit (in which the regulatory sequence, the exon and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of these components into the chromosomal DNA, the expression of the desired endogenous gene is altered.

Altered gene expression, as described herein, encompasses activating (or causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a gene which is not expressed at physiologically significant levels in the cell as obtained. The embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regulation or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

One method by which homologous recombination can be used to increase, or cause, IL-17 like polypeptide production from a cell's endogenous IL-17 like gene involves first

using homologous recombination to place a recombination sequence from a site-specific recombination system (e.g., Cre/loxP, FLP/FRT) (Sauer, Current Opinion In Biotechnology, 5:521-527, 1994; Sauer, Methods In Enzymology, 225:890-900, 1993) upstream (that is, 5' to) of the cell's endogenous genomic IL-17 like polypeptide coding region. A plasmid containing a recombination site homologous to the site that was placed just upstream of the genomic IL-17 like polypeptide coding region is introduced into the modified cell line along with the appropriate recombinase enzyme. This recombinase causes the plasmid to integrate, via the plasmid's recombination site, into the recombination site located just upstream of the genomic IL-17 like polypeptide coding region in the cell line (Baubonis and Sauer, Nucleic Acids Res., 21:2025-2029, 1993; O'Gorman et al., Science, 251:1351-1355, 1991). Any flanking sequences known to increase transcription (e.g., enhancer/promoter, intron, translational enhancer), if properly positioned in this plasmid, would integrate in such a manner as to create a new or modified transcriptional unit resulting in de novo or increased IL-17 like polypeptide production from the cell's endogenous IL-17 like gene.

A further method to use the cell line in which the site specific recombination sequence had been placed just upstream of the cell's endogenous genomic IL-17 like polypeptide coding region is to use homologous recombination to introduce a second recombination site elsewhere in the cell line's genome. The appropriate recombinase enzyme is then introduced into the two-recombination-site cell line, causing a recombination event (deletion, inversion, translocation) (Sauer, Current Opinion In Biotechnology, supra, 1994; Sauer, Methods In Enzymology, supra, 1993) that would create a new or modified transcriptional unit resulting in de novo or increased IL-17 like polypeptide production from the cell's endogenous IL-17 like gene.

An additional approach for increasing, or causing, the expression of IL-17 like polypeptide from a cell's endogenous IL-17 like gene involves increasing, or causing, the expression of a gene or genes (e.g., transcription factors) and/or decreasing the expression of a gene or genes (e.g., transcriptional repressors) in a manner which results in de novo or increased IL-17 like polypeptide production from the cell's endogenous IL-17 like gene. This method includes the introduction of a non-naturally occurring polypeptide (e.g., a polypeptide comprising a site specific DNA binding domain fused to a transcriptional factor domain) into

the cell such that de novo or increased IL-17 like polypeptide production from the cell's endogenous IL-17 like gene results.

The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. The targeting sequence in the DNA construct directs the integration of elements (a)-(d) into a target gene in a cell such that the elements (b)-(d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)-(f) such that the elements of (b)-(f) are operatively linked to the endogenous gene. The targeting sequence is homologous to the preselected site in the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon.

If the sequence of a particular gene is known, such as the nucleic acid sequence encoding an IL-17 like polypeptide presented herein, a piece of DNA that is complementary to a selected region of the gene can be synthesized or otherwise obtained, such as by appropriate restriction of the native DNA at specific recognition sites bounding the region of interest. This piece serves as a targeting sequence(s) upon insertion into the cell and will hybridize to its homologous region within the genome. If this hybridization occurs during DNA replication, this piece of DNA, and any additional sequence attached thereto, will act as an Okazaki fragment and will be incorporated into the newly synthesized daughter strand of DNA. The present invention, therefore, includes nucleotides encoding a IL-17 like molecule, which nucleotides may be used as targeting sequences.

IL-17 like polypeptide cell therapy, e.g., the implantation of cells producing IL-17 like polypeptides, is also contemplated. This embodiment involves implanting cells capable of synthesizing and secreting a biologically active form of IL-17 like polypeptide. Such IL-17 like polypeptide-producing cells can be cells that are natural producers of IL-17 like polypeptides or may be recombinant cells whose ability to produce IL-17 like polypeptides has been augmented by transformation with a gene encoding the desired IL-17 like

polypeptide or with a gene augmenting the expression of IL-17 like polypeptide. Such a modification may be accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered an IL-17 like polypeptide, as may occur with the administration of a polypeptide of a foreign species, it is preferred that the natural cells producing IL-17 like polypeptide be of human origin and produce human IL-17 like polypeptide. Likewise, it is preferred that the recombinant cells producing IL-17 like polypeptide be transformed with an expression vector containing a gene encoding a human IL-17 like polypeptide.

Implanted cells may be encapsulated to avoid the infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in biocompatible, semipermeable polymeric enclosures or membranes that allow the release of IL-17 like polypeptide, but that prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce IL-17 like polypeptides ex vivo, may be implanted directly into the patient without such encapsulation.

Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be routinely accomplished. For example, Baetge et al. (WO95/05452; PCT/US94/09299) describe membrane capsules containing genetically engineered cells for the effective delivery of biologically active molecules. The capsules are biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively linked to promoters that are not subject to down regulation in vivo upon implantation into a mammalian host. The devices provide for the delivery of the molecules from living cells to specific sites within a recipient. In addition, see U.S. Patent Nos. 4,892,538, 5,011,472, and 5,106,627. A system for encapsulating living cells is described in PCT Application no. PCT/US91/00157 of Aebischer et al. See also, PCT Application no. PCT/US91/00155 of Aebischer et al., Winn et al., Exper. Neurol., 113:322-329 (1991), Aebischer et al., Exper. Neurol., 111:269-275 (1991); and Tresco et al., ASAIO. 38:17-23 (1992).

In vivo and in vitro gene therapy delivery of IL-17 like polypeptides is also envisioned. One example of a gene therapy technique is to use the IL-17 like gene (either genomic DNA, cDNA, and/or synthetic DNA) encoding a IL-17 like polypeptide which may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct". The promoter may be homologous or heterologous to the endogenous IL-17 like gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include, DNA molecules designed for site-specific integration (e.g., endogenous sequences useful for homologous recombination), tissue-specific promoter, enhancer(s) or silencer(s), DNA molecules capable of providing a selective advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell-specific binding agents (as, for example, for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as factors to enable vector manufacture.

This gene therapy DNA construct can then be introduced into cells (either ex vivo or in vivo) using viral or non-viral vectors. One means for introducing the gene therapy DNA construct is by means of viral vectors as described herein. Certain vectors, such as retroviral vectors, will deliver the DNA construct to the chromosomal DNA of the cells, and the gene can integrate into the chromosomal DNA. Other vectors will function as episomes, and the gene therapy DNA construct will remain in the cytoplasm.

In yet other embodiments, regulatory elements can be included for the controlled expression of the IL-17 like gene in the target cell. Such elements are turned on in response to an appropriate effector. In this way, a therapeutic polypeptide can be expressed when desired. One conventional control means involves the use of small molecule dimerizers or rapalogs (as described in WO9641865 (PCT/US96/099486); WO9731898 (PCT/US97/03137) and WO9731899 (PCT/US95/03157) used to dimerize chimeric proteins which contain a small molecule-binding domain and a domain capable of initiating biological process, such as a DNA-binding protein or transcriptional activation protein. The dimerization of the proteins can be used to initiate transcription of the IL-17 like gene.

An alternative regulation technology uses a method of storing proteins expressed from the gene of interest inside the cell as an aggregate or cluster. The gene of interest is

expressed as a fusion protein that includes a conditional aggregation domain which results in the retention of the aggregated protein in the endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can be released, however, by administering a drug (e.g., small molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the aggregates or clusters so that the proteins may be secreted from the cell. See, Science 287: 816-817, and 826-830 (2000).

Other suitable control means or gene switches include, but are not limited to, the following systems. Mifepristone (RU486) is used as a progesterone antagonist. The binding of a modified progesterone receptor ligand-binding domain to the progesterone antagonist activates transcription by forming a dimer of two transcription factors which then pass into the nucleus to bind DNA. The ligand binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further described in U.S. 5,364,791; WO9640911, and WO9710337.

Yet another control system uses ecdysone (a fruit fly steroid hormone) which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation domain/DNA-binding domain/ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. 5,514,578; WO9738117; WO9637609; and WO9303162.

Another control means uses a positive tetracycline-controllable transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated tet R-4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, i.e., it binds to a tet operator in the presence of tetracycline) linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Nos. 5,464,758; 5,650,298 and 5,654,168.

Additional expression control systems and nucleic acid constructs are described in U.S. Patent Nos. 5,741,679 and 5,834,186 to Innovir Laboratories Inc.

In vivo gene therapy may be accomplished by introducing the gene encoding an IL-17 like polypeptide into cells via local injection of an IL-17 like nucleic acid molecule or by other appropriate viral or non-viral delivery vectors. Hefti, Neurobiology, 25:1418-1435 (1994). For example, a nucleic acid molecule encoding an IL-17 like polypeptide may be

contained in an adeno-associated virus (AAV) vector for delivery to the targeted cells (e.g., Johnson, International Publication No. WO95/34670; International Application No. PCT/US95/07178). The recombinant AAV genome typically contains AAV inverted terminal repeats flanking a DNA sequence encoding an IL-17 like polypeptide operably linked to functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not limited to, retrovirus, adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an in vivo viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 5,399,346 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells which have been treated in vitro to insert a DNA segment encoding a therapeutic protein. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 5,631,236 involving adenoviral vectors; U.S. Patent No. 5,672,510 involving retroviral vectors; and U.S. 5,635,399 involving retroviral vectors expressing cytokines.

Nonviral delivery methods include, but are not limited to, liposome-mediated transfer, naked DNA delivery (direct injection), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (e.g., gene gun). Gene therapy materials and methods may also include the use of inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to identify transformed cells, negative selection systems and expression control systems (safety measures), cell-specific binding agents (for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 4,970,154 involving electroporation techniques; WO96/40958 involving nuclear ligands; U.S. Patent No. 5,679,559 describing a lipoprotein-containing system for gene delivery; U.S. Patent No. 5,676,954 involving liposome carriers; U.S. Patent No. 5,593,875 concerning methods for calcium phosphate transfection; and U.S. Patent No. 4,945,050 wherein biologically active particles are propelled at cells at a speed

whereby the particles penetrate the surface of the cells and become incorporated into the interior of the cells.

It is also contemplated that IL-17 like gene therapy or cell therapy can further include the delivery of one or more additional polypeptide(s) in the same or a different cell(s). For example, the host cell may be modified to express and release both IL-17 like polypeptide and at least one of the following: IL-1ra, sTNFr Type I, sTNFr Type II, and derivatives thereof; Serine Luekocyte Protease Inhibitor (SLPI), Osteoprotogerin (OPG); and anti-TNF antibodies, anti-IL-1 antibodies, and derivatives thereof.

Such cells may be separately introduced into the patient, or the cells may be contained in a single implantable device, such as the encapsulating membrane described above, or the cells may be separately modified by means of viral vectors.

A means to increase endogenous IL-17 like polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the IL-17 like polypeptide promoter, where the enhancer element(s) can serve to increase transcriptional activity of the IL-17 like gene. The enhancer element(s) used will be selected based on the tissue in which one desires to activate the gene(s); enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a gene encoding a IL-17 like polypeptide is to be "turned on" in T-cells, the lck promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing the IL-17 like polypeptide promoter (and optionally, inserted into a vector and/or 5' and/or 3' flanking sequence(s), etc.) using standard cloning techniques. This construct, known as a "homologous recombination construct", can then be introduced into the desired cells either ex vivo or in vivo.

Gene therapy also can be used to decrease IL-17 like polypeptide expression by modifying the nucleotide sequence of the endogenous promoter(s). Such modification is typically accomplished via homologous recombination methods. For example, a DNA molecule containing all or a portion of the promoter of the IL-17 like gene(s) selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. For example the TATA box and/or the binding site of a transcriptional activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit promoter activity thereby repressing the transcription of the

corresponding IL-17 like gene. The deletion of the TATA box or the transcription activator binding site in the promoter may be accomplished by generating a DNA construct comprising all or the relevant portion of the IL-17 like polypeptide promoter(s) (from the same or a related species as the IL-17 like gene(s) to be regulated) in which one or more of the TATA box and/or transcriptional activator binding site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. The construct will typically contain at least about 500 bases of DNA that correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified. The construct may be introduced into the appropriate cells (either ex vivo or in vivo) either directly or via a viral vector as described herein. Typically, the integration of the construct into the genomic DNA of the cells will be via homologous recombination, where the 5' and 3' DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

Additional Uses of IL-17 Like Nucleic Acids and Polypeptides

Nucleic acid molecules of the present invention (including those that do not themselves encode biologically active polypeptides) may be used to map the locations of the IL-17 like gene and related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and in situ hybridization.

IL-17 like nucleic acid molecules (including those that do not themselves encode biologically active polypeptides), may be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of an IL-17 like DNA or corresponding RNA in mammalian tissue or bodily fluid samples.

Biologically active IL-17 like polypeptides and nucleic acid molecules, may be used to prevent or treat a number of diseases and conditions, including those recited herein.

Biologically active IL-17 like polypeptides and nucleic acid molecules, may also be used in combination with one or more other therapeutic compositions. The IL-17 like polypeptides may be used (simultaneously or sequentially) in combination with one or more cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

Other methods may also be employed where it is desirable to inhibit the activity of one or more IL-17 like polypeptides. Such inhibition may be effected by nucleic acid molecules which are complementary to and hybridize to expression control sequences (triple helix formation) or to IL-17 like mRNA. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of the selected IL-17 like gene(s) can be introduced into the cell. Anti-sense probes may be designed by available techniques using the sequence of IL-17 like polypeptide disclosed herein. Typically, each such antisense molecule will be complementary to the start site (5' end) of each selected IL-17 like gene. When the antisense molecule then hybridizes to the corresponding IL-17 like mRNA, translation of this mRNA is prevented or reduced. Anti-sense inhibitors provide information relating to the decrease or absence of an IL-17 like polypeptide in a cell or organism.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more IL-17 like polypeptides. In this situation, the DNA encoding a mutant polypeptide of each selected IL-17 like polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

In addition, an IL-17 like polypeptide, whether biologically active or not, may be used as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised. Selective binding agents that bind to an IL-17 like polypeptide (as described herein) may be used for in vivo and in vitro diagnostic purposes, including, but not limited to, use in labeled form to detect the presence of IL-17 like polypeptide in a body fluid or cell sample. The antibodies may bind to an IL-17 like polypeptide so as to diminish or block at least one activity characteristic of an IL-17 like polypeptide, or may bind to a polypeptide to increase at least one activity characteristic of an IL-17 like polypeptide (including by increasing the pharmacokinetics of the IL-17 like polypeptide).

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

EXAMPLE 1

PCR was used to screen a panel of 75 human tissue libraries prepared using 2.5 pmol each of primers 2406-26 and 2406-28 and 15ng library cDNA. PCR was performed using Ready-to-Go PCR Beads (Amersham Pharmacia Biotech Catalogue No. #27-9553). PCR was performed in a volume of 25µl. PCR conditions were 94o for 2 minutes; followed by 35 cycles of 94o for 15 seconds; 65o For 30 seconds; 72o for 1 minute; final extension of 72o for 7 minutes and 4o hold. A 238bp band was identified in seven sources with varying signal intensity. The seven libraries were: 1) fetal pancreas-oligo dT library, 2) ovary tumor-oligo dT library, 3) lymphoma-random primed library, 4) normalized fetal tissue-random primed library, 5) testis-oligo dT library, 6) cerebellum-oligo dT library, 7) spinal column-random primed library.

SOURCE	SIGNAL
1) FETAL PANCREAS-DT	+
2) OVARY TUMOR-DT	+++
3) LYMPHOMA-RP	++
4) NORM. FETAL TISSUE-RP	+
5) TESTIS-DT	+
6) CEREBELLUM-DT	++++
7) SPINAL COLUMN-RP	+++

EXAMPLE 2

The libraries used for the screening and RACE were made using the following general procedures.

Total RNA was extracted from the appropriate tissue/cell line using standard RNA extraction procedures and polyA+ RNA was selected from this total RNA using standard procedures known to those skilled in the art. Random primed or oligo(dT) primed cDNA was synthesized from this polyA+ RNA using the procedure in the manual of the Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (Gibco-BRL, Inc., Rockville, MD). The resulting cDNA was digested with appropriate restriction enzymes to create sticky

ends to assist in ligation to a cloning vector. This digested cDNA was ligated into the pSPORT-1 cloning vector, that had been pre-digested with appropriate restriction enzymes. The ligation products were transformed into E. coli using standard techniques known in the art, and transformants were selected on bacterial media plates containing either ampicillin, tetracycline, kanamycin or chloramphenicol, depending upon the specific cloning vector used. The cDNA library consisted of all, or a subset, of these transformants.

PCR was used for both 5'-RACE and 3'-RACE reactions on the seven positive libraries using a touchdown protocol. The 5'-RACE primers used gene specific primer 2406-28 and a library vector (pSPORT-1) primer 1916-83 (5'-GGC TCG TAT GTT GTG TGG AAT TGT GAG CG-3' SEQ ID NO: 5). The 3'-RACE primers used gene specific primer 2406-26 and a library vector primer 1916-80 (5'-TGC AAG GCG ATT AAG TTG GGT AAC GCC AG-3' SEQ ID NO: 6). The PCR conditions were as follows: 94o for 2 minutes; 5 cycles of 94o for 5 seconds and 72o for 2 minutes; 5 cycles of 94o for 5 seconds and 70o for 2 minutes; 25 cycles of 94o for 5 seconds and 68o for 2 minutes; followed by a final extension of 72o for 7 minutes and 4o hold. This reaction used 25ng of each cDNA library, 10pmol of each primer, 200µM dNTP's (final conc.), and a 1x concentration of Clontech's Advantage cDNA Polymerase Mix (Cat# 8417-1) in a 50µl final volume.

A nested PCR reaction was done on the above sample using 5ul of a 1:50 dilution of the first round PCR 5'- and 3'-RACE products, 10 pmol each of a nested gene specific primer and a nested vector primer. (For 5'-nested RACE the gene specific and vector primers were 5'-GCC GAC GGG GAC GTG GAT GAA C-3' (SEQ ID NO: 7) and 5'-CAT GAT TAC GCC AAG CTC TAA TAC GAC TC-3' (SEQ ID NO: 8), respectively. For the 3'-nested RACE the primers were 5'-CTT CGC CGA GTG CCT GTG CAG-3' (SEQ ID NO: 9) and 5'-TCA CGA CGT TGT AAA ACG ACG GCC AGT G-3'(SEQ ID NO: 9), respectively.) The remaining reagents and PCR reaction protocol were identical to those used for the primary RACE reactions.

Ten microliters of the final product from the nested RACE was run on a 1% TBE agarose gel at 5V/cm. Well defined single bands were isolated from the gel and purified using the Qiagen gel extraction kit (Cat#28704) and submitted for sequencing. The sequences of the various RACE products were assembled into a contig which contained the full coding region of the novel IL-17 related protein.

WHAT IS CLAIMED

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2;

(c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO 2; and

(d) a nucleotide sequence complementary to any of (a)-(c).

2. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NO: 2, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NO: 1 wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO 2;

(c) a nucleotide sequence of SEQ ID NO: 1; (a); or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(d) a nucleotide sequence of SEQ ID NO: 1, or (a)-(c) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d), wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2; and

(f) a nucleotide sequence complementary to any of (a)-(c).

3. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(f) a nucleotide sequence of any of (a)-(e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2; and

(h) a nucleotide sequence complementary to any of (a)-(e).

4. A vector comprising the nucleic acid molecule of claims 1, 2, or 3.

5. A host cell comprising the vector of claim 4.

6. The host cell of claim 5 that is a eukaryotic cell.

7. The host cell of claim 5 that is a prokaryotic cell.

8. A process of producing an IL-17 like polypeptide comprising culturing the host cell of claim 5 under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

9. A polypeptide produced by the process of claim 8.

10. The process of claim 8, wherein the nucleic acid molecule comprises promoter DNA other than the promoter DNA for the native IL-17 like polypeptide operatively linked to the DNA encoding the IL-17 like polypeptide.

11. The isolated nucleic acid molecule according to claim 2 wherein the percent identity is determined using the computer program GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

12. A process for identifying candidate inhibitors of IL-17 like polypeptide activity or production comprising exposing a cell according to claims 5, 6, or 7 to the candidate inhibitors, and measuring IL-17 like polypeptide activity or production in said cell, comparing activity of IL-17 like polypeptide in cells exposed to the candidate inhibitor with activity in cells not exposed to the candidate inhibitor.

13. A process for identifying candidate stimulators of IL-17 like polypeptide activity or production comprising exposing a cell according to claims 5, 6 or7 to the candidate stimulators, and measuring IL-17 like polypeptide activity or production in said cell, comparing activity of IL-17 like in cells exposed to the candidate stimulator with activity in cells not exposed to the candidate stimulator.

14. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

15. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the mature amino acid sequence as set forth in SEQ ID NO: 3, comprising a mature amino terminus at residue 5, optionally further comprising an amino-terminal methionine;

(b) an amino acid sequence for an ortholog of SEQ ID NO: 2, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;

(c) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 2, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 as determined using the computer program of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, or the Smith-Waterman algorithm;

(d) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(e) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NO: 2, or at least one of (a)-(c) wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2.

16. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(b) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(c) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2;

(d) the amino acid sequence as set forth in SEQ ID NO: 2 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2; and

(e) the amino acid sequence as set forth in SEQ ID NO: 2, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2.

17. An isolated polypeptide encoded by the nucleic acid molecule of claims 1, 2, or 3.

18. A polypeptide according to claim 15 or 16 wherein the amino acid at position 47 of SEQ ID NO: 2 is leucine, norleucine, isoleucine, valine, methionine, alanine or phenylalanine.

19. A polypeptide according to claim 15 or 16 wherein the amino acid at position 110 of SEQ ID NO: 2 is glutamic acid or aspartic acid.

20. A polypeptide according to claim 15 or 16 wherein the amino acid at position 141 of SEQ ID NO: 2 is tyrosine, thryptophan, phenylalanine, threonine, or serine.

21. A polypeptide according to claim 15 or 16 wherein the amino acid at position 151 of SEQ ID NO: 2 is proline, alanine or glycine.

22. A polypeptide according to claim 15 or 16 wherein the amino acid at position 159 of SEQ ID NO: 2 is cysteine, serine or alanine.

23. A polypeptide according to claim 15 or 16 wherein the amino acid at position 161 of SEQ ID NO: 2 is cysteine, serine or alanine.

24. A polypeptide according to claim 15 or 16 wherein the amino acid at position 164 of SEQ ID NO: 2 is cysteine, serine or alanine.

25. A polypeptide according to claim 15 or 16 wherein the amino acid at position 193 of SEQ ID NO: 2 is cysteine, serine or alanine.

26. A polypeptide according to claim 15 or 16 wherein the amino acid at position 219 of SEQ ID NO: 2 is cysteine, serine or alanine.

27. A polypeptide according to claim 15 or 16 wherein the amino acid at position 221 of SEQ ID NO: 2 is cysteine, serine or alanine.

28. The isolated polypeptide according to claim 15 wherein the percent identity is determined using a computer program selected from the group consisting of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

29. An antibody produced by immunizing an animal with a peptide comprising an amino acid sequence of SEQ ID NO: 2.

30. An antibody or fragment thereof that specifically binds the polypeptide of claims 14, 15, or 16.

31. The antibody of claim 30 that is a monoclonal antibody.

32. A hybridoma that produces a monoclonal antibody that binds to a peptide comprising an amino acid sequence of SEQ ID NO: 2.

33. A method of detecting or quantitating the amount of IL-17 like polypeptide in a sample comprising contacting a sample suspected of containing IL-17 like polypeptide with the anti-IL-17 like antibody or fragment of claims 29, 30, or 31 and detecting the binding of said antibody or fragment.

34. A selective binding agent or fragment thereof that specifically binds at least one polypeptide wherein said polypeptide comprises the amino acid sequence selected from the group consisting of:

the amino acid sequence as set forth in SEQ ID NO: 2; and

a fragment of the amino acid sequence set forth in at least one of SEQ ID NO: 2; or a naturally occurring variant thereof.

35. The selective binding agent of claim 34 that is an antibody or fragment thereof.

36. The selective binding agent of claim 34 that is a humanized antibody.

37. The selective binding agent of claim 34 that is a human antibody or fragment thereof.

38. The selective binding agent of claim 34 that is a polyclonal antibody or fragment thereof.

39. The selective binding agent claim 34 that is a monoclonal antibody or fragment thereof.

40. The selective binding agent of claim 34 that is a chimeric antibody or fragment thereof.

41. The selective binding agent of claim 34 that is a CDR-grafted antibody or fragment thereof.

42. The selective binding agent of claim 34 that is an antiidiotypic antibody or fragment thereof.

43. The selective binding agent of claim 34 which is a variable region fragment.

44. The variable region fragment of claim 43 which is a Fab or a Fab' fragment.

45. A selective binding agent or fragment thereof comprising at least one complementarity determining region with specificity for a polypeptide having the amino acid sequence of SEQ ID NO: 2.

46. The selective binding agent of claim 34 which is bound to a detectable label.

47. The selective binding agent of claim 34 which antagonizes IL-17 like polypeptide biological activity.

48. A method for treating, preventing, or ameliorating a disease, condition, or disorder associated with altered levels of IL-17 like polypeptide comprising administering to a patient an effective amount of a selective binding agent according to claim 34.

49. A selective binding agent produced by immunizing an animal with a polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

50. A hybridoma that produces a selective binding agent capable of binding a polypeptide encoded by the nucleic acid of claims 1, 2, or 3.

51. A composition comprising the polypeptide of claims 14, 15, or 16 and a pharmaceutically acceptable formulation agent.

52. The composition of claim 51 wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, anti-oxidant or combination thereof.

53. The composition of claim 51 wherein the polypeptide comprises the mature amino acid sequence as set forth in SEQ ID NO: 2.

54. A polypeptide comprising a derivative of the polypeptide of claims 14, 15, or 16.

55. The polypeptide of claim 54 which is covalently modified with a water-soluble polymer.

56. The polypeptide of claim 55 wherein the water-soluble polymer is selected from the group consisting of polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, and polyvinyl alcohol.

57. A composition comprising a nucleic acid molecule of claims 1, 2, or 3 and a pharmaceutically acceptable formulation agent.

58. A composition of claim 57 wherein said nucleic acid molecule is contained in a viral vector.

59. A viral vector comprising a nucleic acid molecule of claims 1, 2, or 3.

60. A fusion polypeptide comprising the polypeptide of claims 14, 15, or 16 fused to a heterologous amino acid sequence.

61. The fusion polypeptide of claim 60 wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

62. A method for treating, preventing or ameliorating a medical condition in a mammal resulting from decreased levels of IL-17 like polypeptide comprising administering to a patient the polypeptide of claims 14, 15, or 16 or the polypeptide encoded by the nucleic acid of claims 1, 2, or 3 to said mammal.

63. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject caused by or resulting from abnormal levels of IL-17 like polypeptide comprising:

(a) determining the presence or amount of expression of the polypeptide of claims 14, 15, or 16 or the polypeptide encoded by the nucleic acid molecule of claims 1, 2, or 3 in a sample; and

(b) comparing the level of IL-17 like polypeptide in a biological, tissue or cellular sample from normal subjects or the subject at an earlier time, wherein susceptibility to a pathological condition is based on the presence or amount of expression of the polypeptide.

64. A device, comprising:

(a) a membrane suitable for implantation; and

(b) cells encapsulated within said membrane, wherein said cells secrete a protein of claims 13, 14, or 15;

said membrane being permeable to said protein product and impermeable to materials detrimental to said cells.

65. A device, comprising:

(a) a membrane suitable for implantation; and

(b) the IL-17 like polypeptide encapsulated within said membrane, wherein said membrane is permeable to the polypeptide.

66. A method of identifying a compound which binds to a polypeptide comprising:

(a) contacting the polypeptide of claims 14, 15, or 16 with a compound; and

(b) determining the extent of binding of the polypeptide to the compound.

67. A method of identifying antagonists of IL-17 like polypeptide biological activity comprising:

(a) contacting a small molecule compound with an IL-17 like polypeptide;

(b) detecting the biological activity of an IL-17 like polypeptide in the presence of said small molecule compound; and

(c) comparing the level of IL-17 like polypeptide biological activity in the presence and absence of said small molecule compound.

68. A method of modulating levels of a polypeptide in an animal comprising administering to the animal the nucleic acid molecule of claims 1, 2, or 3.

69. An antagonist of IL-17 like polypeptide activity selected from the group consisting of IL-17 like selective binding agents, small molecules, antisense oligonucleotides, and peptides or derivatives thereof having specificity for IL-17 like polypeptide.

70. A method of reducing cellular production of IL-17 like polypeptide, comprising transforming or transfecting cells with a nucleic acid encoding an antagonist according to claim 69.

71. A method according to claim 70, wherein the antagonist is an antisense reagent, said reagent comprising an oligonucleotide comprising a single stranded nucleic acid sequence capable of binding to IL-17 like mRNA.

72. A transgenic non-human mammal comprising the nucleic acid molecule of claims 1, 2, or 3.

73. A transgenic non-human mammal conprising a disruption of the nucleic acid molecule of claim 1, 2 or 3 wherein the expression of IL-17 like polypeptide is decreased.

74. A diagnostic reagent comprising a detectably labeled polynucleotide encoding the amino acid sequence set out in SEQ ID NO: 2 or a fragment, variant or homolog thereof including allelic variants and spliced variants thereof.

75. The diagnostic reagent of claim 74, wherein said labled polynucleotide is a first-strand cDNA.

76. A method for determine the presence of IL-17 like nucleic acids in a biological sample comprising the steps of:

(a) providing a biological sample suspected of containing IL-17 like nucleic acids;

(b) contacting the biological sample with a diagnostic reagent according to claim 74 under conditions wherein the diagnostic reagent will hybridize with IL-17 like nucleic acids contained in said biological sample;

(c) detecting hybridization between nucleic acid in the biological sample and the diagnostice reagent; and

(d) comparing the level of hybridization between the biological sample and diagnostic reagent with the level of hybridization between a known concentration of IL-17 like nucleic acid and the diagnostic reagent.

77. A method for detecting the presence of IL-17 like nucleic acids in a tissue or cellular sample comprising the steps of:

(a) providing a tissue or cellular sample suspected of containing IL-17 like nucleic acids;

(b) contacting the tissue or cellular sample with a diagnostic reagent according to claim 77 under conditions wherein the diagnostic reagent will hybridize with IL-17 like nucleic acids;

(c) detecting hybridization between IL-17 like nucleic acid in the tissue or cellular sample and the diagnostic reagent; and

(d) comparing the level of hybridization between the tissue or cellular sample and diagnostic reagent with the level of hybridization between a known concentration of IL-17 like nucleic acid and the diagnostic reagent.

78. The method of claim 76 or 77 wherein said polynucleotide molecule is DNA.

79. The method of claim 76 or 77 wherein said polynucleotide molecule is RNA.

Figure 1

Overlap of cDNA (SEQ ID NO: 1) and Predicted Amino Acid Sequence (SEQ ID NO: 2) for the Human IL-17 Like Polypeptide

```
   1  AAGCGCCAGCTGTCACCCCAGTCCAAGAGCTCCAGCAAGGTCACGAGCGTGCTCGGCAAA  60

  61  GCCTCGGATCCCGGCGCCGCCAGCACCAAATCAGGGAAGGCCAGCACGCTGTCTCGGCGG  120

 121  GAGGAGCTGCTGAAACAGCTGAAGGCCGTGGAGGATGCTATTGCACGCAAGCGGGCCAAG  180
                        *  R  P  W  R  M  L  L  H  A  S  G  P  R  -

 181  ATCCCCGGGAAAGCATAGGCCGTGCCCCGACCGGACTGGACGCATTTTTATACATAGGCT  240
       S  P  G  K  H  R  P  C  P  D  R  T  G  R  I  F  I  H  R  L  -

 241  CCTCCCCGGCCTCCTGTTTCTGACCTGGCTGCACACATGCCTGGCCCACCATGACCCCTC  300
       L  P  G  L  L  F  L  T  W  L  H  T  C  L  A  H  H  D  P  S  -

 301  CCTCAGGGGGCACCCCCACAGTCACGGTACCCCACACTGCTACTCGGCTGAGGAACTGCC  360
       L  R  G  H  P  H  S  H  G  T  P  H  C  Y  S  A  E  E  L  P  -

 361  CCTCGGCCAGGCCCCCCCACACCTGCTGGCTCGAGGTGCCAAGTGGGGGCAGGCTTTGCC  420
       L  G  Q  A  P  P  H  L  L  A  R  G  A  K  W  G  Q  A  L  P  -

 421  TGTAGCCCTGGTGTCCAGCCTGGAGGCAGCAAGCCACAGGGGGAGGCACGAGAGGCCCTC  480
       V  A  L  V  S  S  L  E  A  A  S  H  R  G  R  H  E  R  P  S  -

 481  AGCTACGACCCAGTGCCCGGTGCTGCGGCCGGAGGAGGTGTTGGAGGCAGACACCCACCA  540
       A  T  T  Q  C  P  V  L  R  P  E  E  V  L  E  A  D  T  H  Q  -

 541  GCGCTCCATCTCACCCTGGAGATACCGTGTGGACACGGATGAGGACCGCTATCCACAGAA  600
       R  S  I  S  P  W  R  Y  R  V  D  T  D  E  D  R  Y  P  Q  K  -

 601  GCTGGCCTTCGCCGAGTGCCTGTGCAGAGGCTGTATCGATGCACGGACGGGCCGCGAGAC  660
       L  A  F  A  E  C  L  C  R  G  C  I  D  A  R  T  G  R  E  T  -

 661  AGCTGCGCTCAACTCCGTGCGGCTGCTCCAGAGCCTGCTGGTGCTGCGCCGCCGGCCCTG  720
       A  A  L  N  S  V  R  L  L  Q  S  L  L  V  L  R  R  R  P  C  -

 721  CTCCCGCGACGGCTCGGGGCTCCCCACACCTGGGGCCTTTGCCTTCCACACCGAGTTCAT  780
       S  R  D  G  S  G  L  P  T  P  G  A  F  A  F  H  T  E  F  I  -

 681  CCACGTCCCCGTCGGCTGCACCTGCGTGCTGCCCCGTTCAGTGTGACCGCCGAGGCCGTG  840
       H  V  P  V  G  C  T  C  V  L  P  R  S  V  *

 841  GGGCCCCTAGACTGGACACGTGTGCTCCCCAGAGGGCACCCCCTATTTATGTGTATTTAT  900

 901  TGTTATTTATATGCCTCCCCCAACACTACCCTTGGGGTCTGGGCATTCCCCGTGTCTGGA  960

 961  GGACAGCCCCCCACTGTTCTCCTCATCTCCAGCCTCAGTAGTTGGGGGTAGAAGGAGCTC 1020

1021  AGCACCTCTTCCAGCCCTTAAAGCTGCAGAAAAGGTGTCACACGGCTGCCTGTACCTTGG 1280
```

Figure 1B

1081 CTCCCTGTCCTGCTCCCGGCTTCCCTTACCCTATCACTGGCCTCAGGCCCCCGCAGGCTG 1140

1141 CCTCTTCCCAACCTCCTTGGAAGTACCCCTGTAAATG 1177

Figure 2

Predicted Amino Acid Sequence with Amino Terminal Signal Peptide Sequence for the Human IL-17 Like Polypeptide (SEQ ID NO: 3)

```
  1  MLLHASGPRS PGKHRPCPDR TGRIFIHRLL PGLLFLTWLH TCLAHHDPSL
 51  RGHPHSHGTP HCYSAEELPL GQAPPHLLAR GAKWGQALPV ALVSSLEAAS
101  HRGRHERPSA TTQCPVLRPE EVLEADTHQR SISPWRYRVD TDEDRYPQKL
151  AFAECLCRGC IDARTGRETA ALNSVRLLQS LLVLRRRPCS RDGSGLPTPG
201  AFAFHTEFIH VPVGCTCVLP RSV*
```

Figure 3

Overlap of Human IL-17 Like Amino Acid Sequence (SEQ ID NO: 3) with Known Human IL-17 Family Member (SEQ ID NO: 4)

```
Zhvt-002560 MLLHASGPRSPGKHRPCPDRTGRIFIHRLLPGLLFLTWLHTCLAHHDPSL 50
                                                 :  |  |  |    |:
Human IL-17 ..................................MDWPHNLLFLLTISI 15

Zhvt-002560 RGHPHSHGTPHCYSAEELPLGQAPPHLLARGAKWGQALPVALVSSLEAAS 100
                  | |   | .   ||  |  || |      .|. ||| :.  .
Human IL-17 F...LGLGQPR..SPKSKRKGQGRPGPLAPGP...HQVPLDLVSRMKPYA 57

Zhvt-002560 HRGRHERPSATTQCPVLRPEEVLEAD.........THQRSISPWRYRVDT 141
                :||        .   |. :           ...||:||| | :.
Human IL-17 RMEEYERNIEEMVAQLRNSSELAQRKCEVNLQLWMSNKRSLSPWGYSINH 107

Zhvt-002560 DEDRYPQKLAFAECLCRGCIDARTGRETAALNSVRLLQSLLVLRRRPCSR 191
            |  | |  |  | ||| ||:.  | .|  .: || .  | . .||| |
Human IL-17 DPSRIPVDLPEARCLCLGCVNPFTMQEDRSMVSVPVF.SQVPVRRRLCPP 156

Zhvt-002560 DGSGLPTPGAFAFHTEFIHVPVGCTCVLPRSV* 224
                  |  |         : |||||:
Human IL-17 P....PRTGPCRQRAVMETIAVGCTCIF      180
```

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gcctcggatc ccggcgccgc cagcaccaaa tcagggaagg ccagcacgct gtctcggcgg 120

gaggagctgc tgaaacagct ga agg ccg tgg agg atg cta ttg cac gca agc 172
Arg Pro Trp Arg Met Leu Leu His Ala Ser
1 5 10

ggg cca aga tcc ccg gga aag cat agg ccg tgc ccc gac cgg act gga 220
Gly Pro Arg Ser Pro Gly Lys His Arg Pro Cys Pro Asp Arg Thr Gly
15 20 25

cgc att ttt ata cat agg ctc ctc ccc ggc ctc ctg ttt ctg acc tgg 268
Arg Ile Phe Ile His Arg Leu Leu Pro Gly Leu Leu Phe Leu Thr Trp
30 35 40

ctg cac aca tgc ctg gcc cac cat gac ccc tcc ctc agg ggg cac ccc 316
Leu His Thr Cys Leu Ala His His Asp Pro Ser Leu Arg Gly His Pro
45 50 55

cac agt cac ggt acc cca cac tgc tac tcg gct gag gaa ctg ccc ctc 364
His Ser His Gly Thr Pro His Cys Tyr Ser Ala Glu Glu Leu Pro Leu
60 65 70

ggc cag gcc ccc cca cac ctg ctg gct cga ggt gcc aag tgg ggg cag 412
Gly Gln Ala Pro Pro His Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln
75 80 85 90

gct ttg cct gta gcc ctg gtg tcc agc ctg gag gca gca agc cac agg 460
Ala Leu Pro Val Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His Arg
95 100 105

```
ggg agg cac gag agg ccc tca gct acg acc cag tgc ccg gtg ctg cgg   508
Gly Arg His Glu Arg Pro Ser Ala Thr Thr Gln Cys Pro Val Leu Arg
            110                 115                 120

ccg gag gag gtg ttg gag gca gac acc cac cag cgc tcc atc tca ccc   556
Pro Glu Glu Val Leu Glu Ala Asp Thr His Gln Arg Ser Ile Ser Pro
        125                 130                 135

tgg aga tac cgt gtg gac acg gat gag gac cgc tat cca cag aag ctg   604
Trp Arg Tyr Arg Val Asp Thr Asp Glu Asp Arg Tyr Pro Gln Lys Leu
    140                 145                 150

gcc ttc gcc gag tgc ctg tgc aga ggc tgt atc gat gca cgg acg ggc   652
Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile Asp Ala Arg Thr Gly
155                 160                 165                 170

cgc gag aca gct gcg ctc aac tcc gtg cgg ctg ctc cag agc ctg ctg   700
Arg Glu Thr Ala Ala Leu Asn Ser Val Arg Leu Leu Gln Ser Leu Leu
                175                 180                 185

gtg ctg cgc cgc cgg ccc tgc tcc cgc gac ggc tcg ggg ctc ccc aca   748
Val Leu Arg Arg Arg Pro Cys Ser Arg Asp Gly Ser Gly Leu Pro Thr
            190                 195                 200

cct ggg gcc ttt gcc ttc cac acc gag ttc atc cac gtc ccc gtc ggc   796
Pro Gly Ala Phe Ala Phe His Thr Glu Phe Ile His Val Pro Val Gly
        205                 210                 215

tgc acc tgc gtg ctg ccc cgt tca gtg tgaccgccga ggccgtgggg         843
Cys Thr Cys Val Leu Pro Arg Ser Val
    220                 225

cccctagact ggacacgtgt gctccccaga gggcaccccc tatttatgtg tatttattgt 903

tatttatatg cctccccccaa cactaccctt ggggtctggg cattccccgt gtctggagga 963

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  1               5                  10                  15

Lys His Arg Pro Cys Pro Asp Arg Thr Gly Arg Ile Phe Ile His Arg
            20                  25                  30

Leu Leu Pro Gly Leu Leu Phe Leu Thr Trp Leu His Thr Cys Leu Ala
        35                  40                  45

His His Asp Pro Ser Leu Arg Gly His Pro His Ser His Gly Thr Pro
    50                  55                  60
```

```
His Cys Tyr Ser Ala Glu Glu Leu Pro Leu Gly Gln Ala Pro Pro His
 65                  70                  75                  80

Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln Ala Leu Pro Val Ala Leu
                85                  90                  95

Val Ser Ser Leu Glu Ala Ala Ser His Arg Gly Arg His Glu Arg Pro
            100                 105                 110

Ser Ala Thr Thr Gln Cys Pro Val Leu Arg Pro Glu Glu Val Leu Glu
        115                 120                 125

Ala Asp Thr His Gln Arg Ser Ile Ser Pro Trp Arg Tyr Arg Val Asp
    130                 135                 140

Thr Asp Glu Asp Arg Tyr Pro Gln Lys Leu Ala Phe Ala Glu Cys Leu
145                 150                 155                 160

Cys Arg Gly Cys Ile Asp Ala Arg Thr Gly Arg Glu Thr Ala Ala Leu
                165                 170                 175

Asn Ser Val Arg Leu Leu Gln Ser Leu Leu Val Leu Arg Arg Arg Pro
            180                 185                 190

Cys Ser Arg Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe Ala Phe
        195                 200                 205

His Thr Glu Phe Ile His Val Pro Val Gly Cys Thr Cys Val Leu Pro,
    210                 215                 220

Arg Ser Val
225
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Cys Pro Asp Arg Thr Gly Arg Ile Phe Ile His Arg Leu Leu Pro Gly
             20                  25                  30

Leu Leu Phe Leu Thr Trp Leu His Thr Cys Leu Ala His His Asp Pro
         35                  40                  45

Ser Leu Arg Gly His Pro His Ser His Gly Thr Pro His Ala Glu Glu
     50                  55                  60

Leu Pro Leu Gly Gln Ala Pro Pro His Leu Leu Ala Arg Gly Ala Lys
 65                  70                  75                  80

Trp Gly Gln Ala Leu Pro Val Ala Leu Val Ser Ser Leu Glu Ala Ala
                85                  90                  95

Ser His Arg Gly Arg His Glu Arg Pro Ser Ala Thr Thr Gln Cys Pro
            100                 105                 110

Val Leu Arg Pro Glu Glu Val Leu Glu Ala Asp Thr His Gln Arg Ser
        115                 120                 125
```

```
Ile Ser Pro Trp Arg Tyr Arg Val Asp Thr Asp Glu Asp Arg Tyr Pro
    130                 135                 140

Gln Lys Leu Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile Asp Ala
145                 150                 155                 160

Arg Thr Gly Arg Glu Thr Ala Ala Leu Asn Ser Val Arg Leu Leu Gln
                165                 170                 175

Ser Leu Leu Val Leu Arg Arg Arg Pro Cys Ser Arg Asp Gly Ser Gly
            180                 185                 190

Leu Pro Thr Pro Gly Ala Phe Ala Phe His Thr Glu Phe Ile His Val
        195                 200                 205

Pro Val Gly Cys Thr Cys Val Leu Pro Arg Ser Val
    210                 215                 220
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  1               5                  10                  15

Gly Leu Gly Gln Pro Arg Ser Pro Lys Ser Lys Arg Lys Gly Gln Gly
            20                  25                  30

Arg Pro Gly Pro Leu Ala Pro Gly Pro His Gln Val Pro Leu Asp Leu
        35                  40                  45

Val Ser Arg Met Lys Pro Tyr Ala Arg Met Glu Glu Tyr Glu Arg Asn
    50                  55                  60

Ile Glu Glu Met Val Ala Gln Leu Arg Asn Ser Ser Glu Leu Ala Gln
65                  70                  75                  80

Arg Lys Cys Glu Val Asn Leu Gln Leu Trp Met Ser Asn Lys Arg Ser
                85                  90                  95

Leu Ser Pro Trp Gly Tyr Ser Ile Asn His Asp Pro Ser Arg Ile Pro
            100                 105                 110

Val Asp Leu Pro Glu Ala Arg Cys Leu Cys Leu Gly Cys Val Asn Pro
        115                 120                 125

Phe Thr Met Gln Glu Asp Arg Ser Met Val Ser Val Pro Val Phe Ser
    130                 135                 140

Gln Val Pro Val Arg Arg Arg Leu Cys Pro Pro Pro Arg Thr Gly Pro
145                 150                 155                 160

Cys Arg Gln Arg Ala Val Met Glu Thr Ile Ala Val Gly Cys Thr Cys
                165                 170                 175

Ile Phe
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